

FIGURE 1A

100 bp scale
"N" region
Nde/Sma Ndel lused to SmaI
Transcription start site

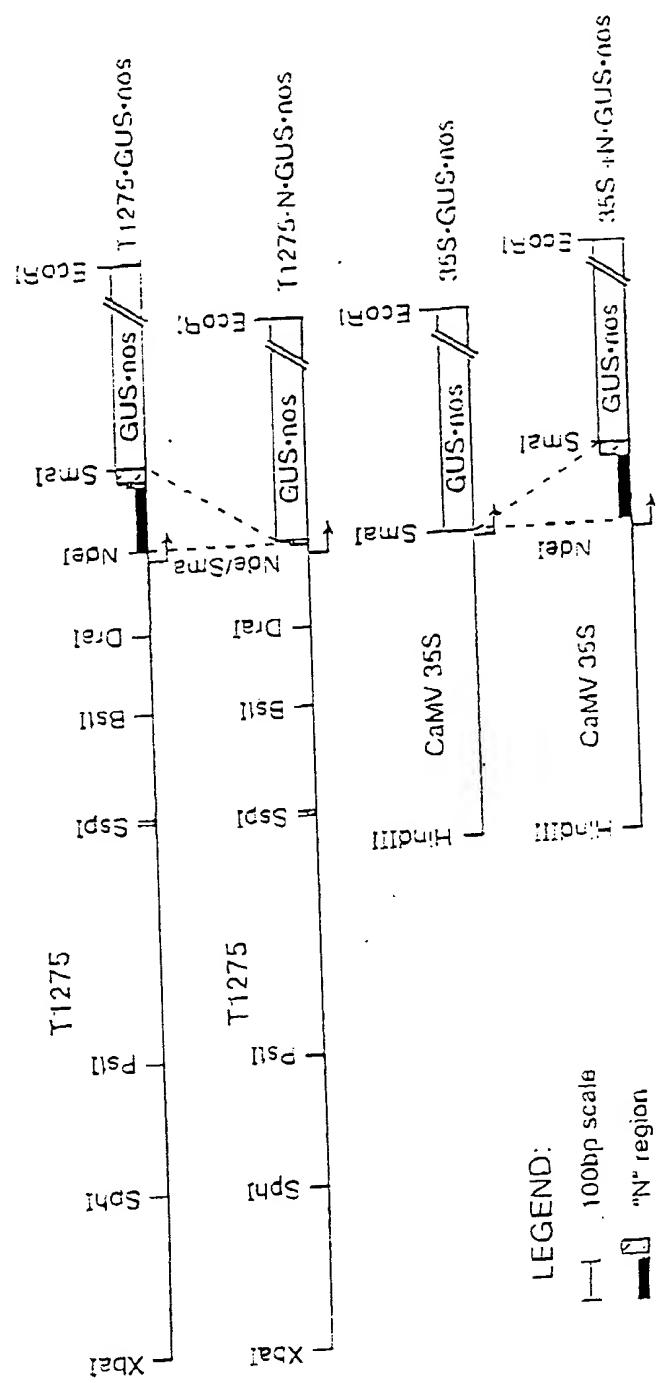


FIGURE 1B

	10	20	30	40	50
cCUP	1	-----	-----	-----	-----
Rent1.	1	-----	A	-----	ATTGTGAGCG GGATAACAT
RENT2.	1	-----	-----	AT	GTTGTGCGA ATTGTGAGCG GGATAACAT
RENT3.	1	TT	-----	-----	-----
RENT5.	1	-----	-----	-----	GGA ATTGTGAGCG G-ATAACAT
RENT7.	1	TTTATGCTTC CGGCTCGTAT	GTTGTGCGA	ATTGTGAGCG	G-ATAACAT

	60	70	80	90	100	
cCUP	51	-----	-----	-----	-----	
100	51	TTCACACAGG	AAACAGCTAT	GACCATGATT	ACGCCAAGCT TTTAATACGA	
Rent1.	100	51	TTCACACAGG	AAACAGCTAT	GACCATGATT	ACGCCAAGCT CT-AATACGA
RENT2.	100	51	-----	-----	-----	
RENT3.	100	51	-----	-----	-----	
RENT5.	100	51	TTCACACAGG	AAACAGCTAT	GACCATGATT	ACGCCAAGCT CT-AATANGA
RENT7.	100	51	TTCACACAGG	AAACAGCTAT	GACCATGATT	ACGCCAAGCT CT-AATACGA

	110	120	130	140	150	
cCUP	101	-----	-----	ITA	TAATTACAAA ATTGATTCTA GTATTTAA	
150	101	CTCACTATAG	GGAAAGCTTA	TAATTACAAA	ATTGATTCTA GTATTTAA	
RENT1.	150	101	CTCACTATAG	GGAAAGCTTA	TAATTACAAA	ATTGATTCTA GTATTTAA
RENT2.	150	101	CTCACTATAG	GGAAAGCTTA	TAATTACAAA	ATTGATTCTA GTATTTAA
RENT3.	150	101	-----	-----	GATTCTA GTTTTTAA	
RENT5.	150	101	CTCACTATAG	GGAAAGCTTA	TAATTACAAA	TTTGATTCTA GTATTTAA
RENT7.	150	101	CTCACTATAG	GGAAAGCTTA	TAATTACAAA	ATTGATTCTA GTACTTTAA

FIGURE 1C1

cCUP	160	170	180	190	200		
200	151	TTTAATGCTT	ATACATTATT	AATTAATTTA	GTACTTTCAA	TTTGTGTTCA	
RENT1.	200	151	TTTAATATTT	TTACATTATT	AATTAATTTA	GAAGTTTTAA	TTTTTTTCAA
RENT2.	200	151	TTTAATATTT	ATACATTATT	AATTAACCTTA	GTACTTTCAA	TTCGTTTCAA
RENT3.	200	151	TTTAATATTT	ATACATTATT	AATTAATTTA	GTTCTTTCAA	TTTGTGTTCA
RENT5.	200	151	TTTAATATTT	ATACATTATT	AATTAATTTA	GTACTTTCAA	TTTGTGTTCA
RENT7.	200	151	TTTAATATTT	ATACATTATT	AATTAATTTA	GCACTTTCAA	TTTATTTTCAA

cCUP	210	220	230	240	250		
250	201	GAAATTATTT	TACTATTTTT	TATAAAATAA	AAGGGAGAAA	ATGGCTATTT	
RENT1.	250	201	GAAATCATT	TACTATTTTT	-ATAAAAACAA	AAAGGGAAAA	GTGGTTATTT
RENT2.	250	201	AAAATTATTT	TACTATTTTT	TGTAATAAA	AAGGGAGAAA	ATGGCTATTT
RENT3.	250	201	GAAATTATTT	TACTATTTTT	TATAAAATAA	AAGGGAGAAA	ATGGCTATTT
RENT5.	250	201	GAAATCATT	TACTATGGTT	TATAAAATAA	AAGGGAGAAA	ATGGCTATTT
RENT7.	250	201	GAAACCATT	TACTATTTTT	TATAAAATAA	AAGGGACAAA	ATGGCTATTT

cCUP	260	270	280	290	300		
300	251	AAATACTAGC	-CTATTTAT	TTCAATTATA	GCCTAAAATC	AG-CCCCAAT	
RENT1.	300	251	AAATACTAGC	CCTATTTCAT	TTCAATTATA	GCCTAAAATC	AGCCCC-AAT
RENT2.	300	251	AAATACTAGC	CCTATTTAT	TTCAATTATA	GCCTAAAATC	AGCCCCCAAT
RENT3.	300	251	AAATACCAGC	CCTATTTAT	TTCAATTATA	ACCTAAAATC	AGCCCC-AGT
RENT5.	300	251	AAATACTAGC	CCTATTTAT	TTCAATTATA	GCCTAAAATC	AGCCCC-AAT
RENT7.	300	251	AAATACCAAC	ACTATTTAT	TTCAATTATA	GCCTAAAATC	AAACCC-AAT

FIGURE 1C2

	310	320	330	340	350
CCUP	350	301	TAGCCCCAAT TTCAAATTCA AATGGTCCAG CCCAATTCCCT AAA-TAACCC		
RENT1.	350	301	TAACCCCCAAT TCCAAATTCA AACGGGCCAG CCCAATTCCCT AAAATGACCC		
RENT2.	350	301	TAACCCCCAAT TTCAAATTCA AATGGGACAG CCCAATTCCCT AAAATAACCC		
RENT3.	350	301	TAGCCCC--- -----A AACGGGCCAT CCCAATTCCCT AAAATAACTC		
RENT5.	350	301	TAACCCCTAT TTCAATTCA AACGGGCTAG CCCAGTTCCCT AAAATAACCC		
RENT7.	350	301	TAACCCCC--- -----A AACGGGCCAG CCCAATTCCCT AAAACAAACCC		
	350				

	360	370	380	390	400
CCUP	400	351	ACCCCTAACCC C----- -----GCCCGG TTTCCCTTT TGATCCAGGC		
RENT1.	400	351	GCTCCTAACCC CGCTTTCCA ACCCGCCCGG TTTCCCTTT TGATCCAGGC		
RENT2.	400	351	GCCCTAACCC CTCTTATCCA ACCCACCCGA TTTCCCTTT TGATCCAGGT		
RENT3.	400	351	GCCCTAACCC CGCTTATCCA ACCCGCCCGG TTCCC-CTTT TGATCCAGGC		
RENT5.	400	351	TCCCCCTAACCC CGCTTATCCA ACCCGCCCTG TTTCCCTTT TGATCCAGGC		
RENT7.	400	351	GCCCTAACCC CGCTTATCCA ACCCGCCCGA TTCCCTCTTT TGATCCAGGC		
	400				

	410	420	430	440	450
CCUP	450	401	CGTTGATCAT TTTGATCAAC GCCCAGAATT TCCCCTTTC CTTTTTAAT		
RENT1.	450	401	TGTTGATCAT TTTGATCAAC GGCCAGAATT TCCCCTTTC --TTTTTAAT		
RENT2.	450	401	TGTTGATCAT TTTGATCAAC GACCAGAATT TCCCCTTTC TGTTTTTAAT		
RENT3.	450	401	CGTTGATCAT TTTGATCAAC GACCAGAATT TCCCCTTTC -TTTTTTAAT		
RENT5.	450	401	CGTTGATCAT TTTGATCAAC GACCAAAATT TCCCCTTT-C CTTTTTTAAT		
RENT7.	450	401	CGTTGATCAT TTTGATCAAC GGCCAGAATT TCCCCTTTC -TTTTTTCAT		
	450				

FIGURE 1C3

	460	470	480	490	500
TCUP					
RENT1.	500				
RENT2.	500				
RENT3.	500				
RENT5.	500				
RENT7.	500				

451 TCCCAAACAC C-CCTAACTC TATCCCATT CTCACCAACC GCCACATATG
 451 TCCCAAACAC CCCCCAACCT TATCCCGTTT CTCACCAACC GCCAGATCT-
 451 TCCCAAACAC CCCCCAACCC TATCCCATT CTCACCAACC GCCAGATCT-
 451 TCCCAAACAC CGCC-AAACC TATCCCATT CTCACCAACC GCCAGATCTA
 451 TCCCAAACAC CCCC-AACCC TATCCCATT CTCACCAACC GCCAGATCT-
 451 TCCCAAACAC CCCC-AAACC TATCCCATT CTCACCAACC GCCAGATCTA

	510	520	530	540	550
TCUP					
RENT1.	550				
RENT2.	550				
RENT3.	550				
RENT5.	550				
RENT7.	550				

501 AATCCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCCTAA CCCTAGCAGC
 501 -ATCCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCCTAA CCCTAGCAGC
 501 -ATCCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCCTAA CCCTAGCAGC
 501 T--CCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCCTAA CCCTAGCAGC
 501 -ATCCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCCTAA CCCTAGCAGC
 501 T--CCTCTTA TCTCTCAAAC TCTCTCGAAC CTTCCCCCTAA CCCTAGCAGC

	560	570	580	590	600
TCUP					
RENT1.	600				
RENT2.	600				
RENT3.	600				
RENT5.	600				
RENT7.	600				

551 CTCTCATCAT CCTCACCTCA AAACCCACCG GAATACATGG CCTCTCAAGC
 551 CTCTCATCAT CCTCACCTCA AAACCCACCG GCCACCATGG CCTCTAGAG-
 551 CTCTCATCAT CCTCACCTCA AAACCCACCG GCCACCATGG CCTCTAGAG-
 551 CTCTCATCAT CCTCACCTCA AAACCCACCG GCCACCATGG CCTCTAGAGG
 551 CTCTCATCAT CCTCACCTCA AAACCCACCG GCCACCATGG CCTCTAGAG-
 551 CTCTCATCAT CCTCACCTCA AAACCCACCG GCCACCATGG CCTCTAGAGG

FIGURE 1C4

610	620	630	640	650
601 CGTGGAAACC TTATACTCAC CTCCCTTGC TCTTACAGTA CTC-GGCCGT				
650				
RENT1.				
650				
RENT2.				
650				
RENT3.				
650				
RENT5.				
650				
RENT7.				
650				
	660	670	680	690
	700			
651 CGACCGCGGT ACCCGGG...				
651 C-----CTNAA...				
651 TTAT-GTG--CGTC...				
651 CCGNCCTGNN NNNNN-C...				
651 -----TCC-----				
651 -----				

FIGURE 1C5

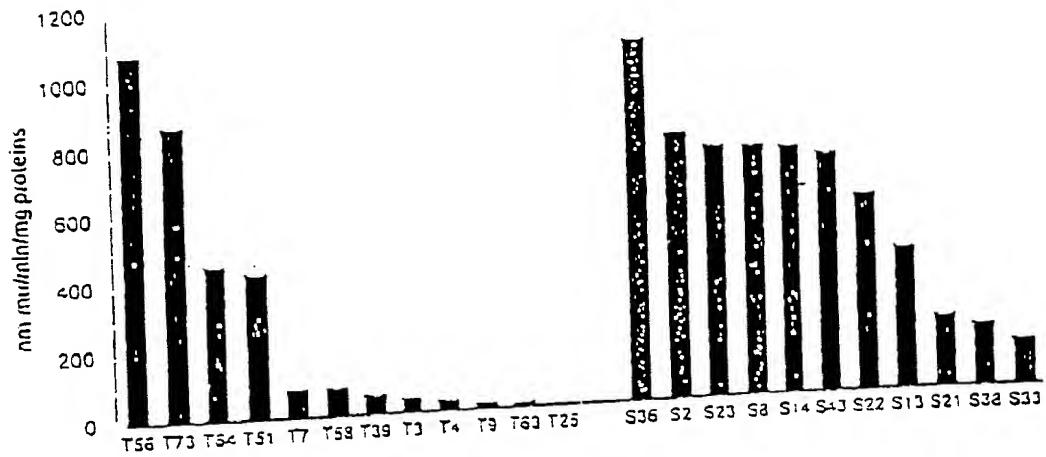


FIGURE 2A

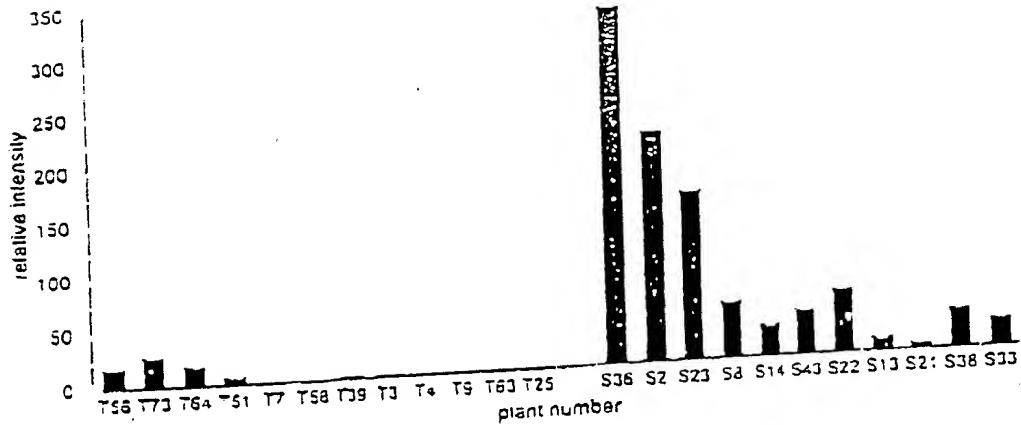
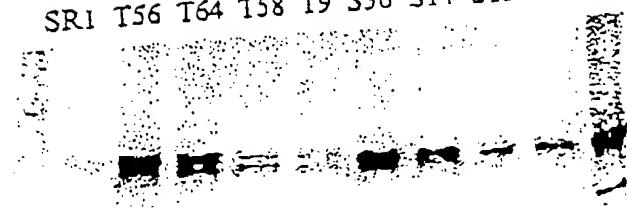


FIGURE 2B

SR1 T56 T64 T58 T9 S36 S14 S13 S33 E.c.



-109

-80

-51

FIGURE 2C

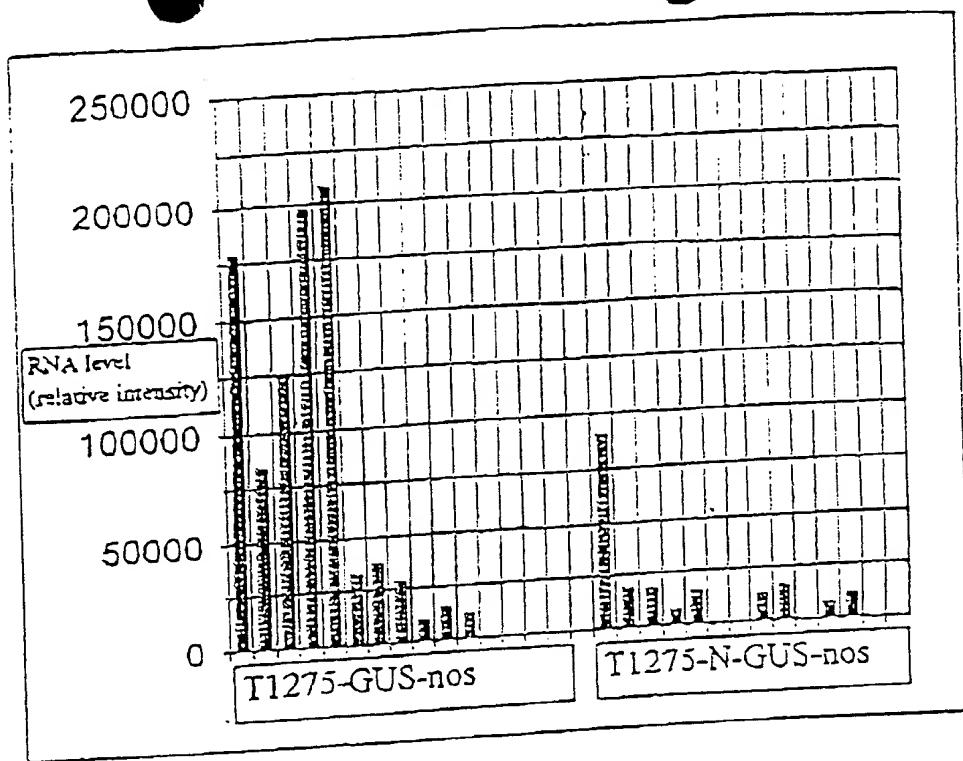


FIGURE 3A

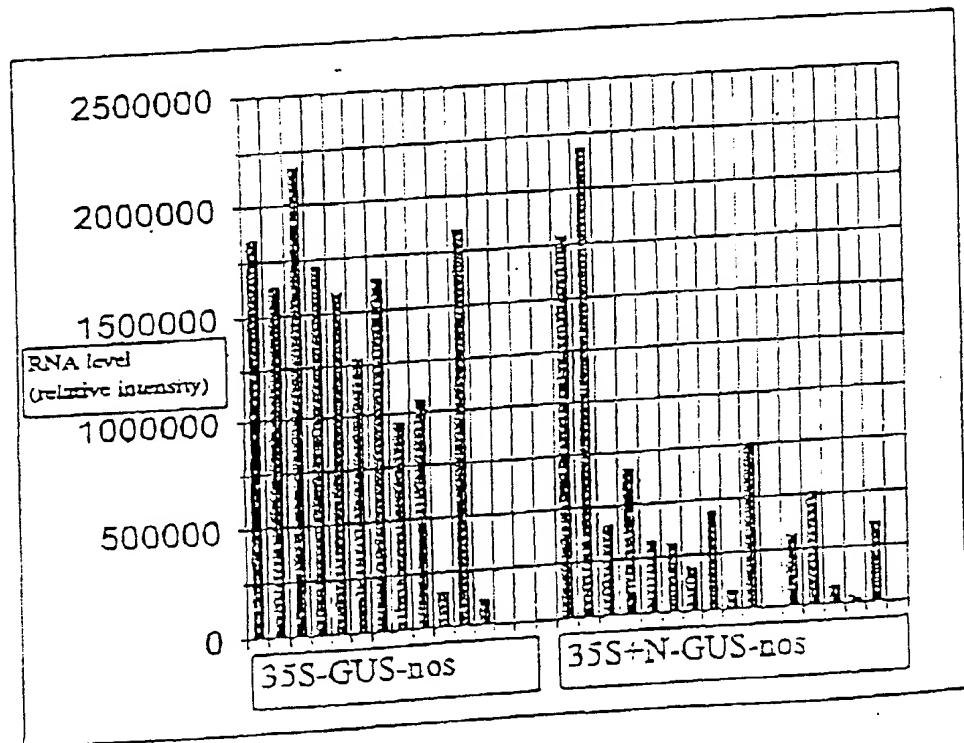


FIGURE 3B

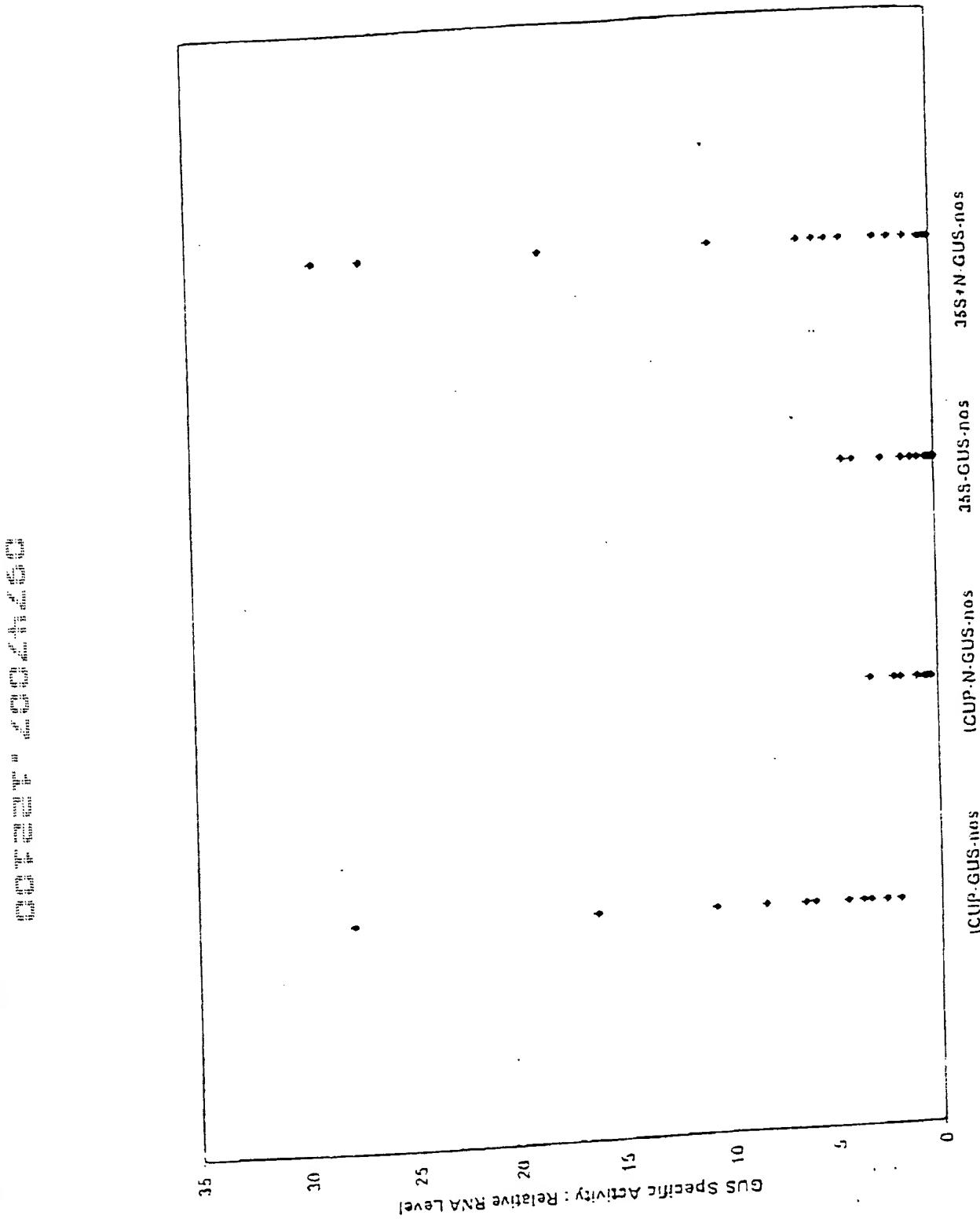


FIGURE 3C

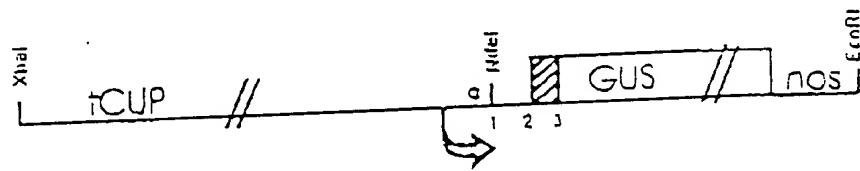
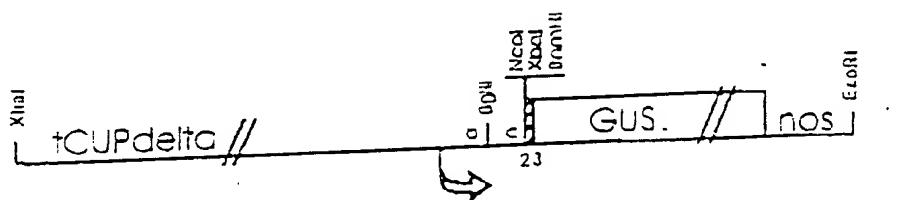
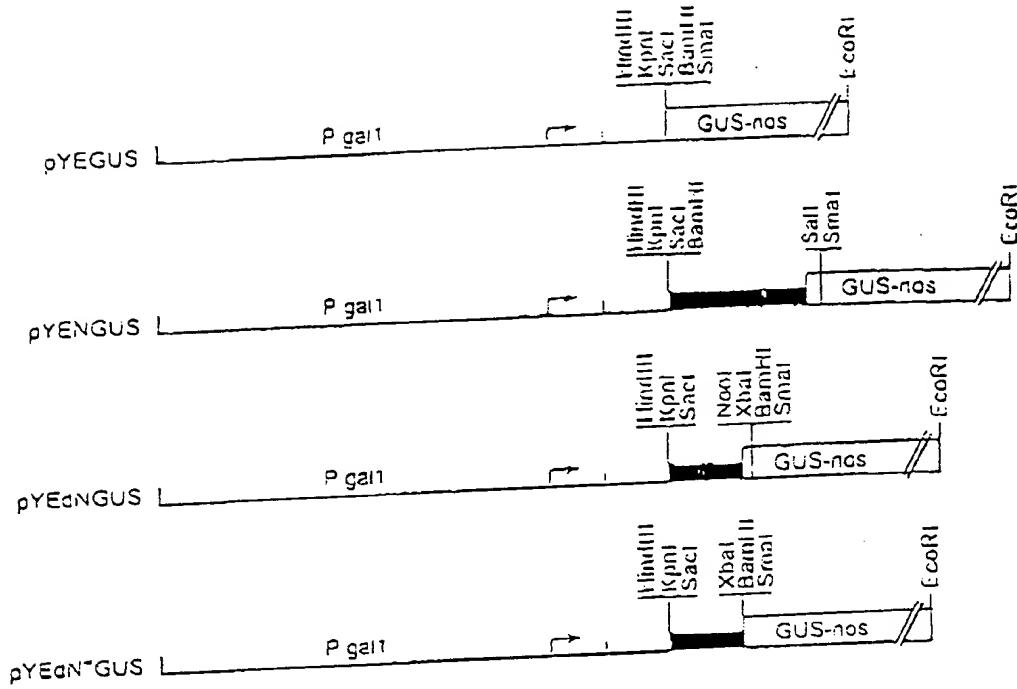


FIGURE 4A



a Ncol position -20 1
 ACA TAT GAA
 " "
 b SgII position +29 2
 ACA GAT CT
 c Ncol position 0 3
 CC ACC ATG GCC TCT AGA GGA TCC CCG CGT GGT CAG TCC CTT ATG
 (CUP initiation site GAA TAC ATG G / ... tCUP leader ... / CCG CGT GGT CAG TCC CTT ATG
 Kazak consensus CC ACC ATG G

FIGURE 4B



LEGEND:

- Vector sequence
- N, dN, or dN'
- UidA reporter gene
- ↑ Start of transcription
- 50bp

FIGURE 5A

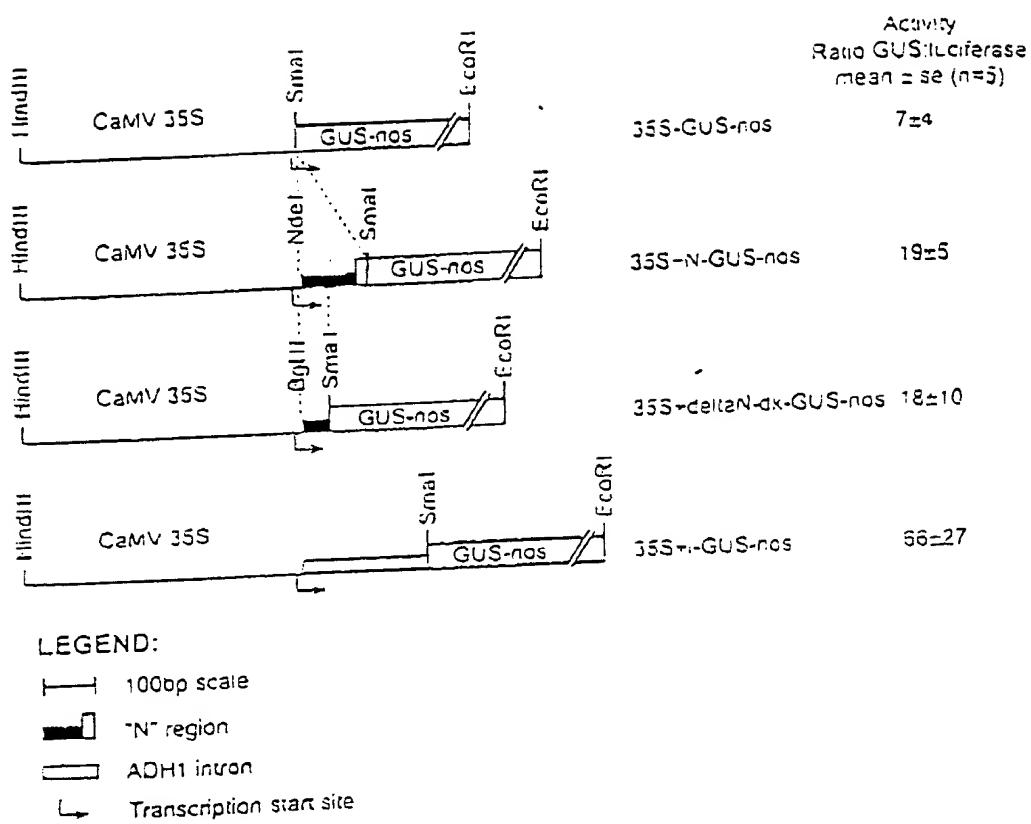


FIGURE 5B

White Spruce Callus
Transient Analysis 14/12/99

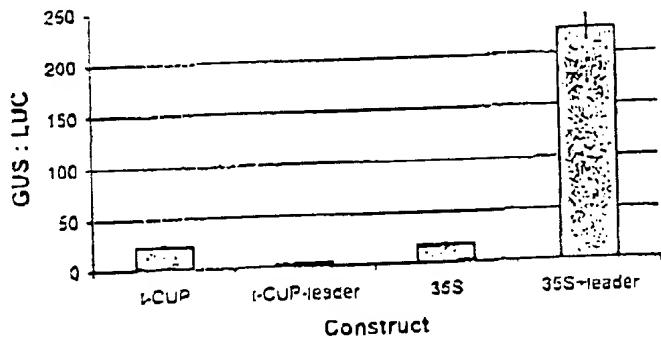


FIGURE 5C

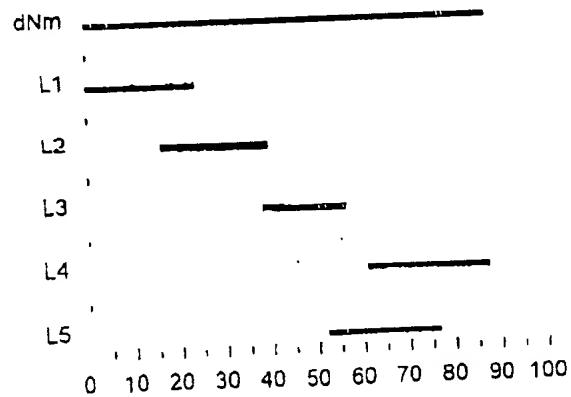


FIGURE 6A

Linker 1: GGATCTATCCTCTTATCTCTCAA
Linker 2: ATCTCTCAAACCTCTCTCGAACCTT
Linker 3: TTCCCCCTAACCCCTAGCAG
Linker 4: ATCATCCTCACCTCAAAACCCACC
Linker 5: AGCCTCTCATCATCCTCACCTCAA

FIGURE 6B

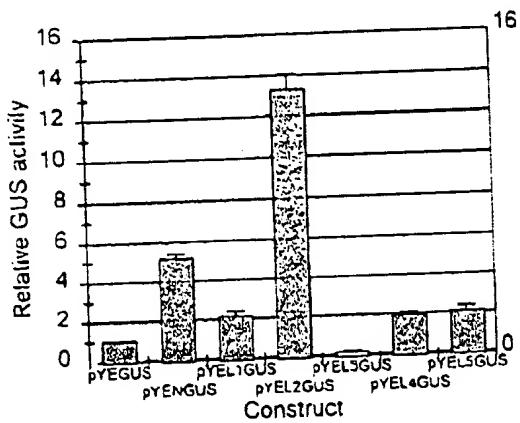


FIGURE 6C

L2 AUCUCUCAA**ACUCUCUC**GAACCUU
 L2C AUCUCUCAA**ACUCUCU**
 L2R **ACUCUCUC**GAACCUU

FIGURE 6D

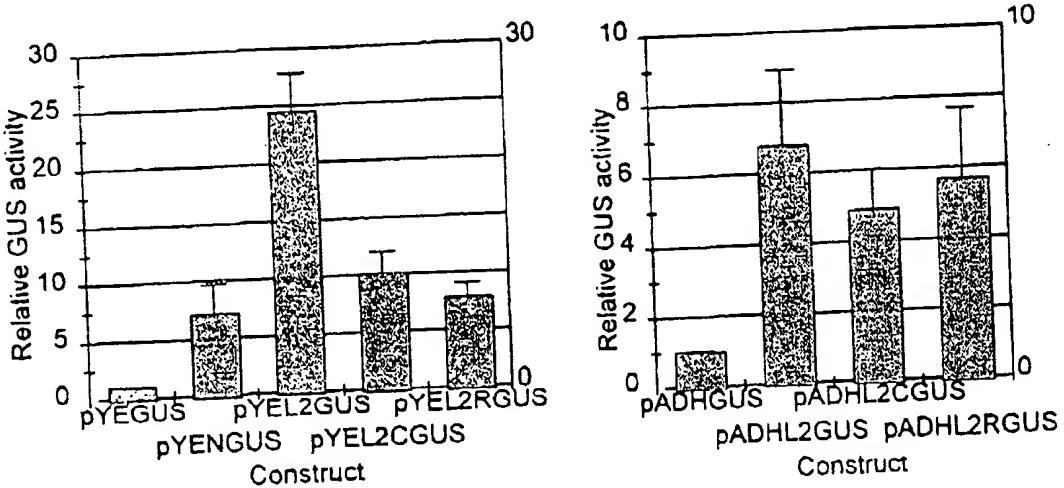


FIGURE 6E

L2	A TCT CTC AAA CTC TCT CGA ACC TT
SCAN1	a AGA ctc aaa ctc tct cga acc tt
SCAN2	a tct GAG aaa ctc tct cga acc tt
SCAN3	a tct ctc GGG ctc tct cga acc tt
SCAN4	a tct ctc aaa GAG tct cga acc tt
SCAN5	a tct ctc aaa ctc AGA cga acc tt
SCAN6	a tct ctc aaa ctc tct GCT acc tt
SCAN7	a tct ctc aaa ctc tct cga GAG tt

FIGURE 6F

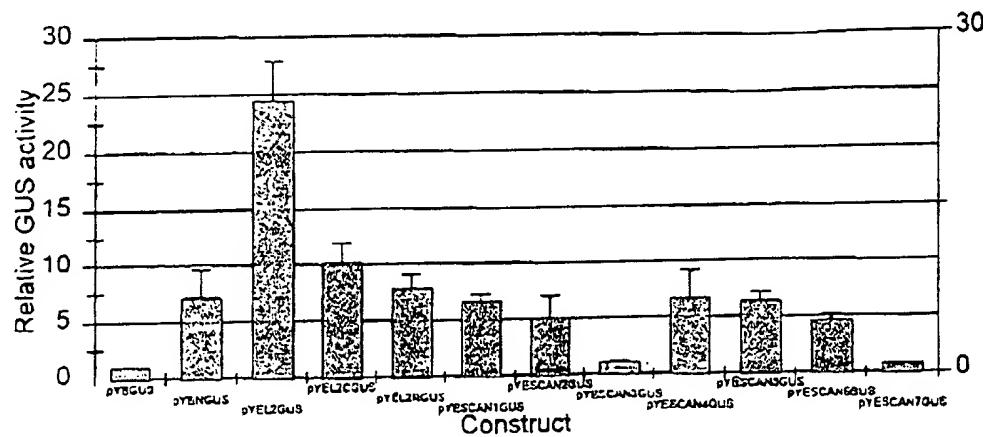


FIGURE 6G

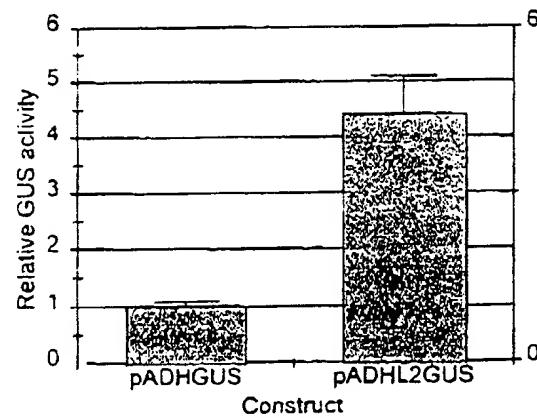
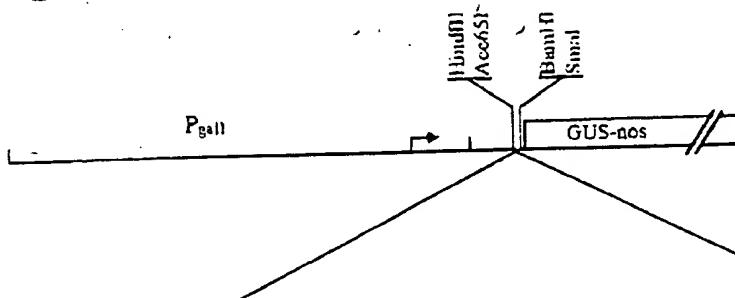


FIGURE 6H



pYEGUS	(no sequence)
pYEL1GUS	GGATCTATCCTCTTATCTCTCAA
pYEL2GUS	ATCTCTCAAACCTCTCTCGAACCTT
pYEL3GUS	TTCCCCCTAACCCCTAGCAG
pYEL4GUS	ATCATCCTCACCTCAAAACCCACC
pYEL5GUS	AGCCTCTCATCATCCTCACCTCAA
pYEL2CGUS	ATCTCTCAAACCTCTCT
pYEL2RGUS	ACTCTCTCGAACCTT
pYELMGUS	ACTCTCTC
pYESCAN1GUS	AAGACTCAAACCTCTCTCGAACCTT
pYESCAN2GUS	ATCTGAGAAACTCTCTCGAACCTT
pYESCAN3GUS	ATCTCTCGGGCTCTCTCGAACCTT
pYESCAN4GUS	ATCTCTCAAAGAGTCTCGAACCTT
pYESCAN5GUS	ATCTCTCAAACCTCAGACGAACCTT
pYESCAN6GUS	ATCTCTCAAACCTCTGCTACCTT
pYESCAN7GUS	ATCTCTCAAACCTCTCGAGAGTT
pYEB1-12GUS	ATCTCTCAAACCTATCTCGAAACTT
pYEB7-12GUS	ATCTCTCAAACCTCTCTCAAACCTT
pYEL2D1GUS	ATCTCTC---CTCTCTCGAACCTT
pYEL2D2GUS	ATCTCTCAAACCTCTCTCGA---TT
pYEL2D3GUS	ATCTCTC---CTCTCTCGA---TT
pYE2L2GUS	ATCTCTCAAACCTCTCTCGAACCTTCTCAAACCTCTCTCGAACCTT

LEGEND:

- Vector sequence
- GUS reporter gene
- Start of transcription

FIGURE 6I

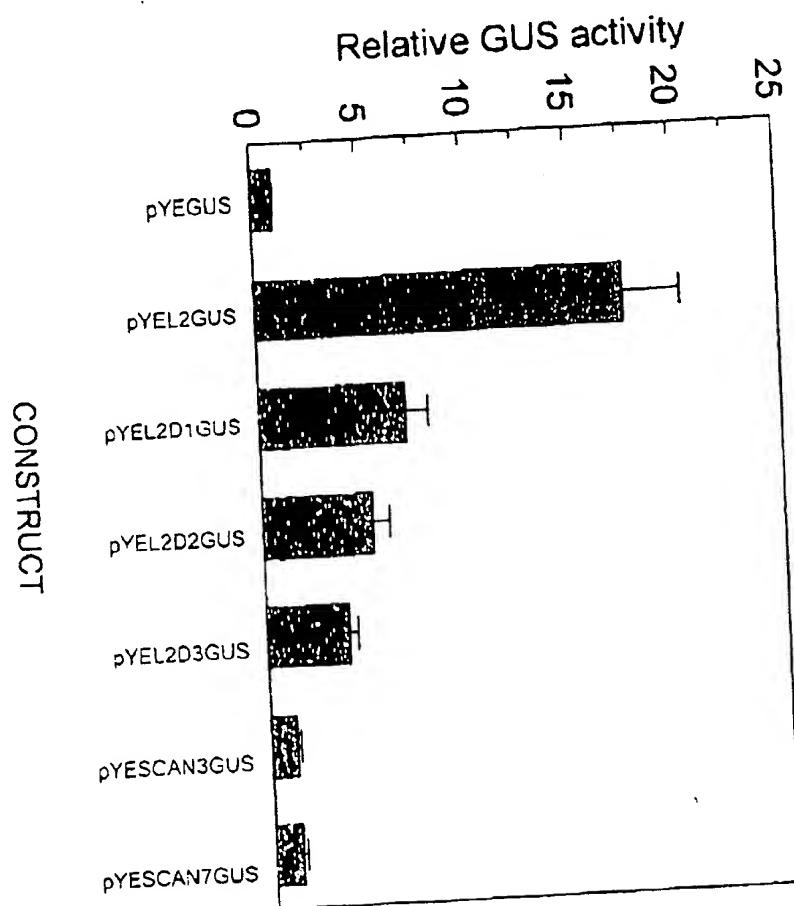


FIGURE 6J

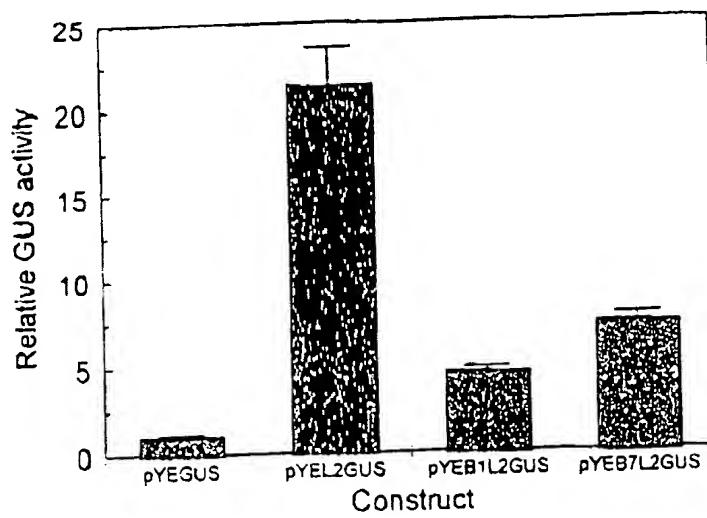


FIGURE 6K

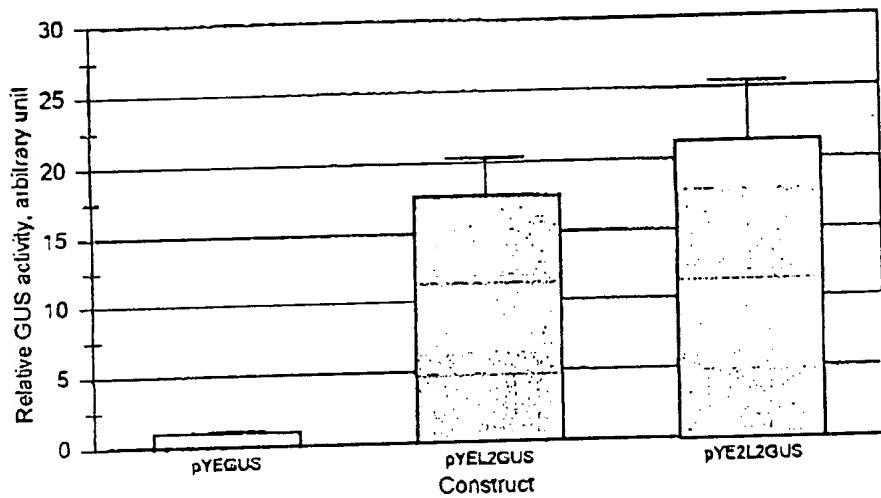
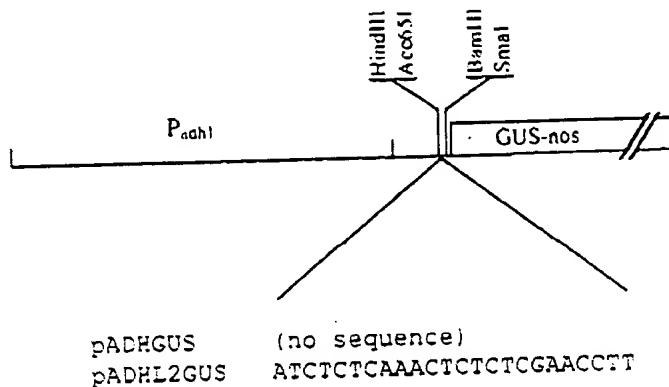


FIGURE 6L



LEGEND:

- Vector sequence
- GUS reporter gene

FIGURE 6M

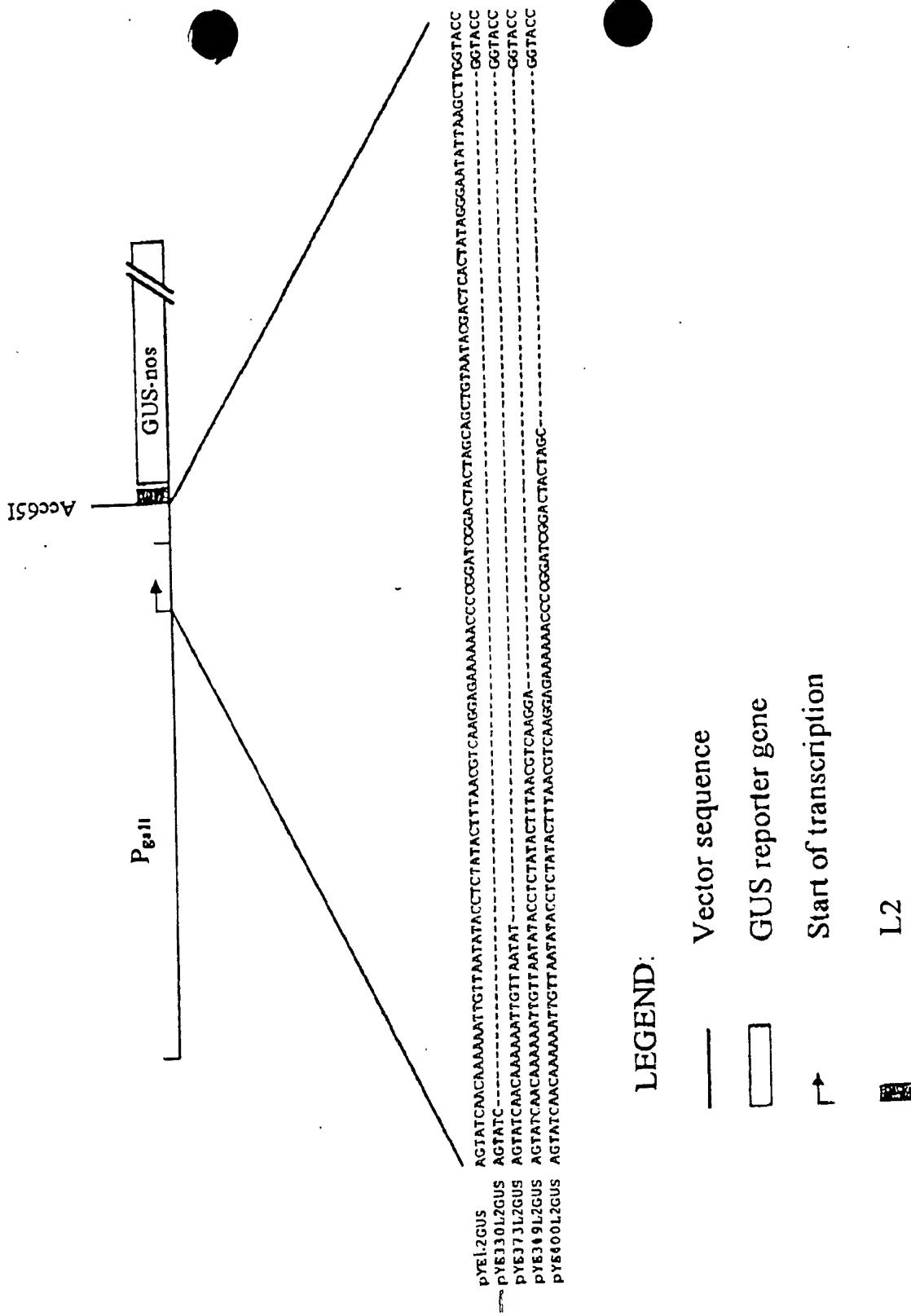


FIGURE 6N

Analysis of GUS activity in yeast expression system



FIGURE 60

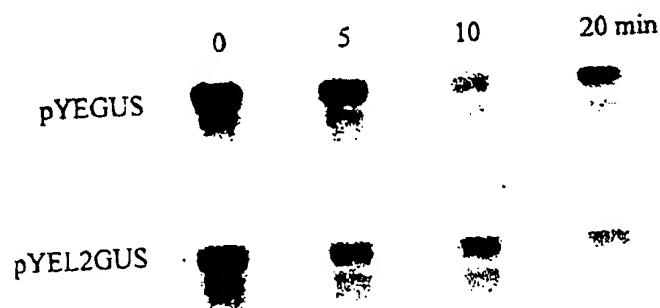


FIGURE 6P.1

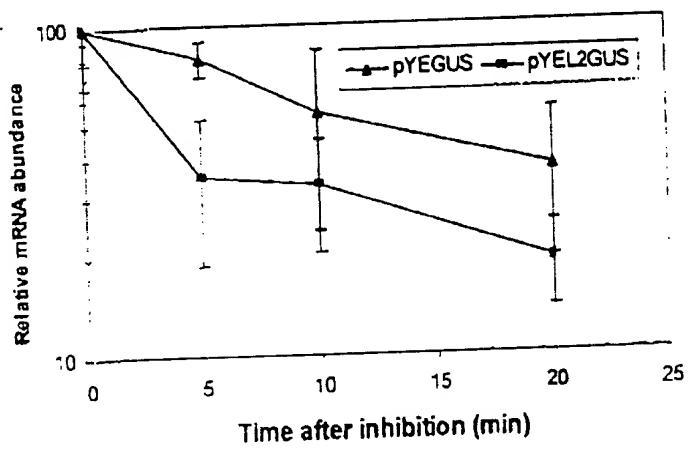


FIGURE 6P.2

Enhanced tCUP Versions 1-3

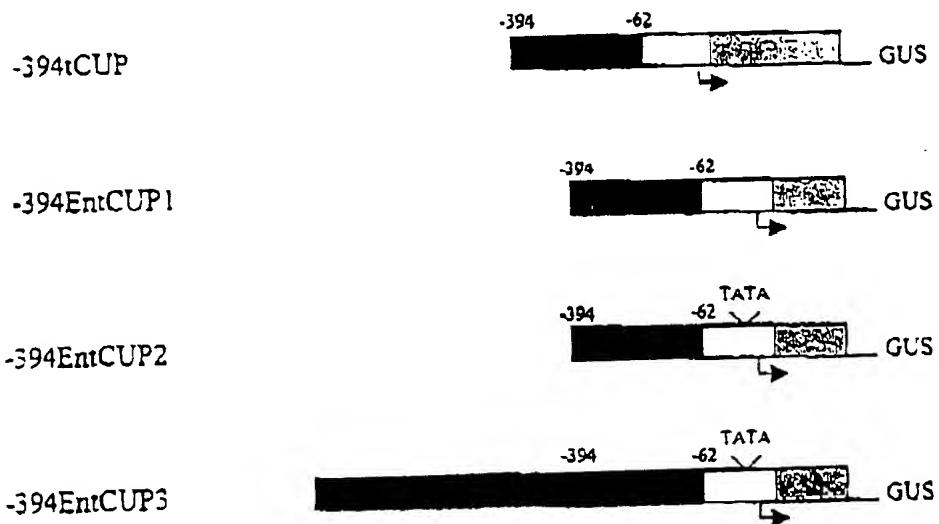
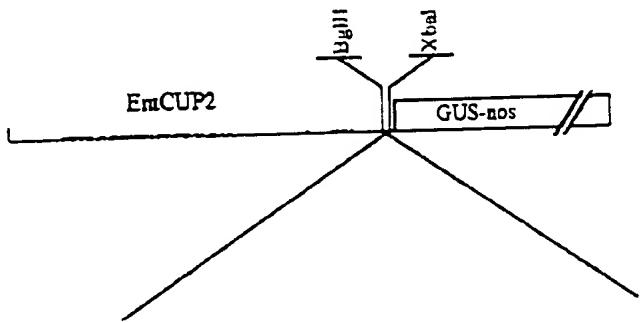


FIGURE 7A.1

30



pUC_cCUP2(-N)GUS
 pUC_cCUP2L1GUS
 pUC_cCUP2L2GUS
 pUC_cCUP2L3GUS
 pUC_cCUP2L4GUS
 pUC_cCUP2L5GUS
 pUC_cCUP2SCAN3GUS
 pUC_cCUP2SCAN7GUS
 pUC_cCUP2-2XL2GUS

(no sequence)
 GGATCTATCCCTCTTATCTCTCAA
 ATCTCTCAAACCTCTCTCGAACCTT
 TTCCCCCTAACCCCTAGCAG
 ATCATCTCACCTCAAACCCRC
 AGCCTCTCATCATCTCTCACCTCRA
 ATCTCTGGGGCTCTCTCGAACCTT
 ATCTCTCAAACCTCTCGAGAGTT
 ATCTCTCAAACCTCTCGAACCTTCTCTCGAACCTT

FIGURE 7A.2

Pooled Expression of GUS Enhanced by L Series Fragments and enh-ICUP2 in Tobacco Transient Assay

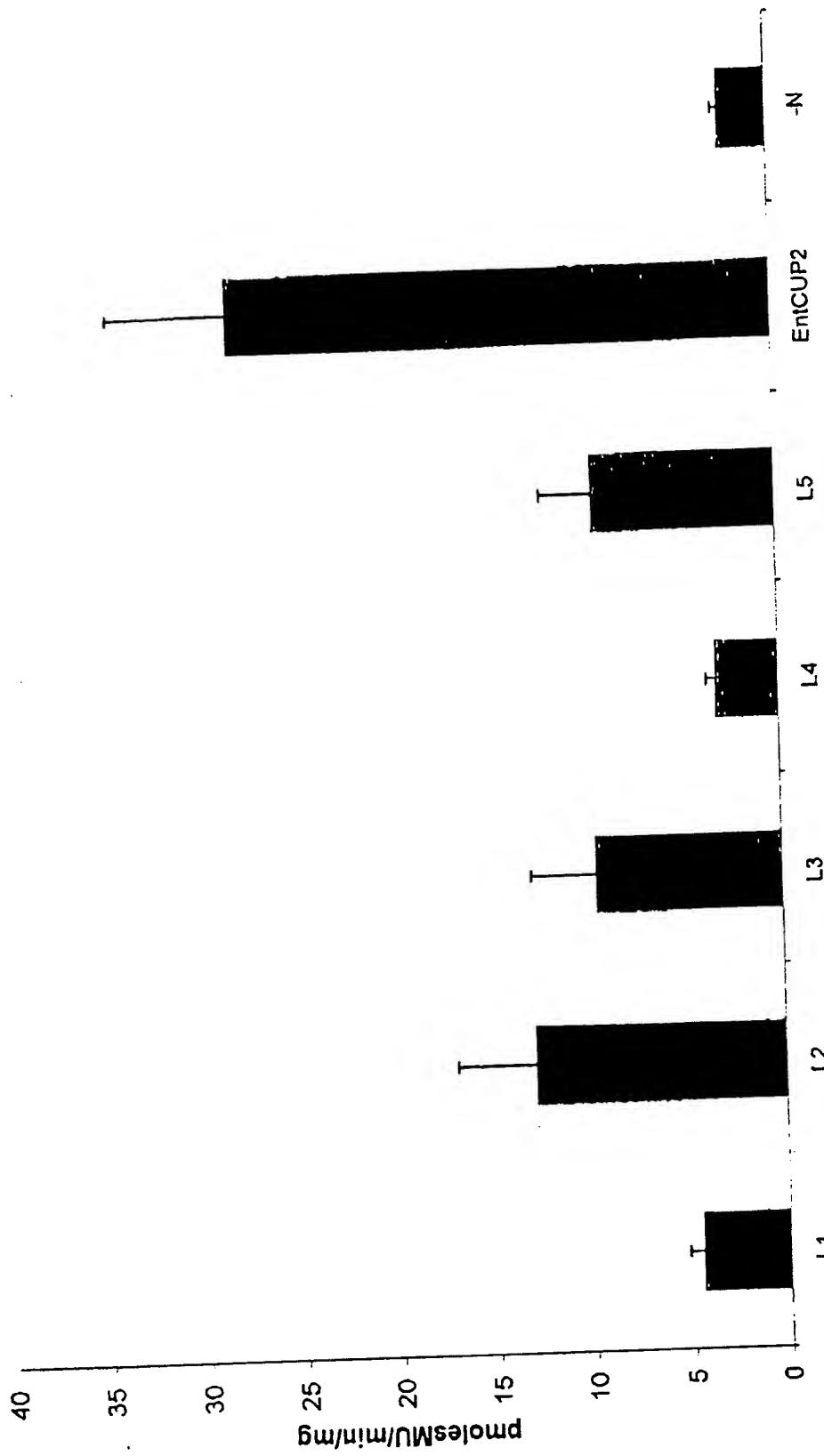


FIGURE 7B

Evaluation of tCUP leader element, L1, L2, L3, L4, and L5 on transient GUS gene expression in alfalfa suspension culture

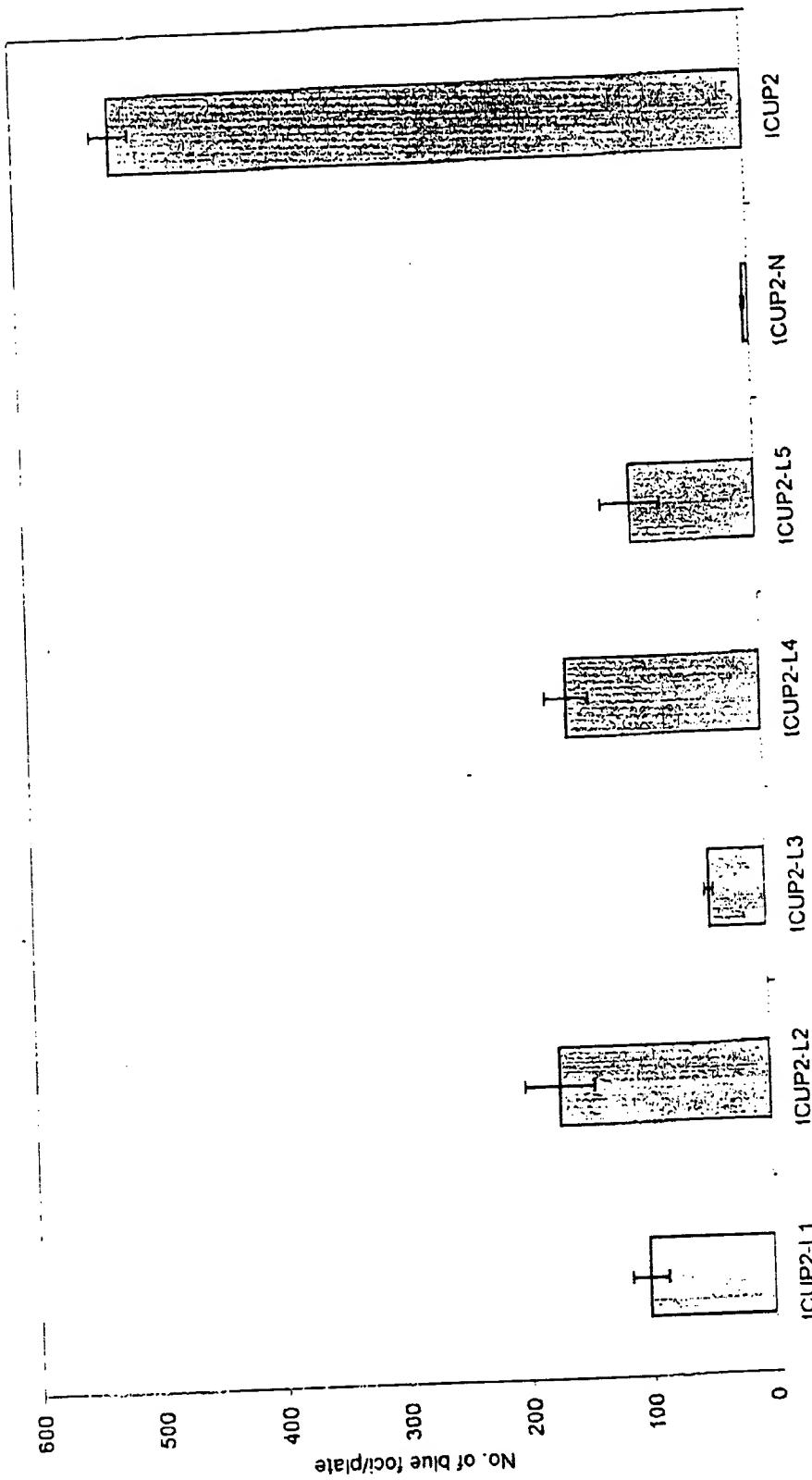


FIGURE 7C

Evaluation of tCUP leader elements, L1, L2, L3, L4, and L5 on transient GUS gene expression in white spruce callus

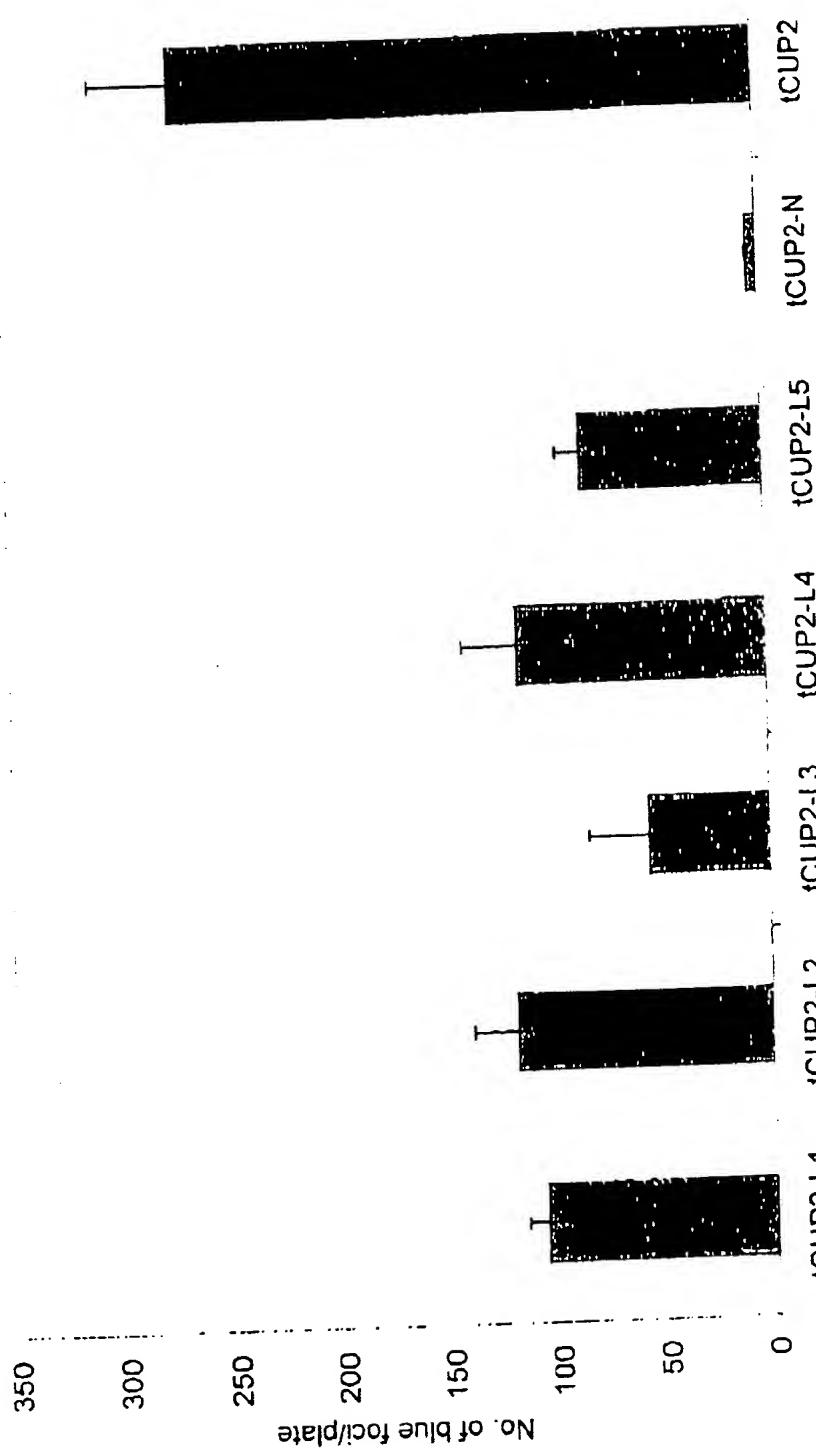
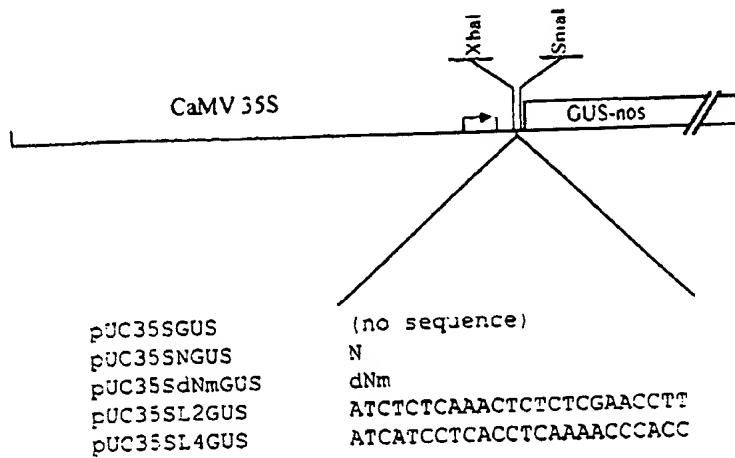


FIGURE 7D
Construct



LEGEND:

- Vector sequence
- GUS reporter gene
- Start of transcription

FIGURE 8A

Stable Transformation of *Arabidopsis* with GUS enhanced by
L-series constructs and the 35S promoter

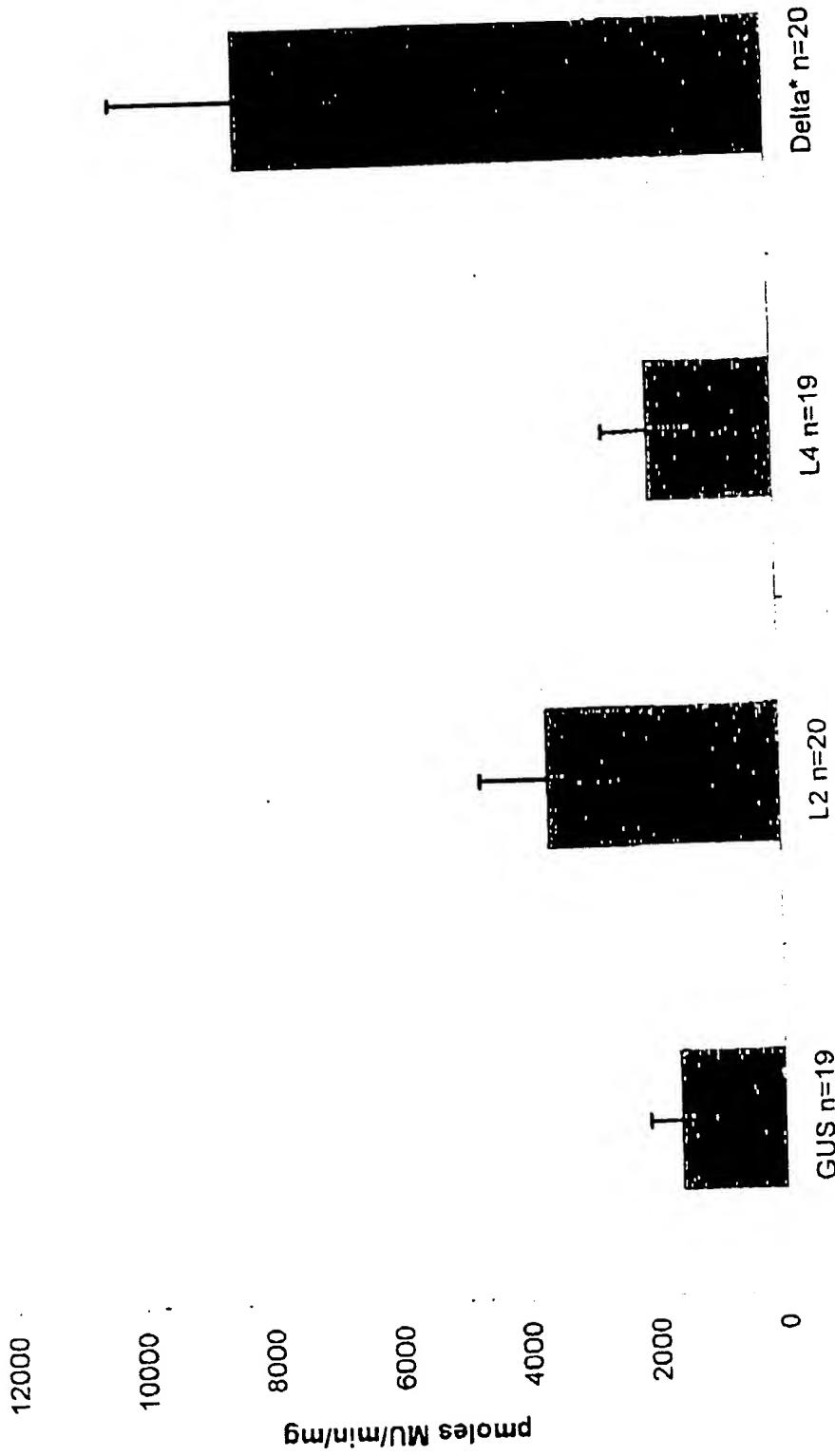


FIGURE 8B

Effect of L2 & L4 on 35S Pea Protoplast Expression

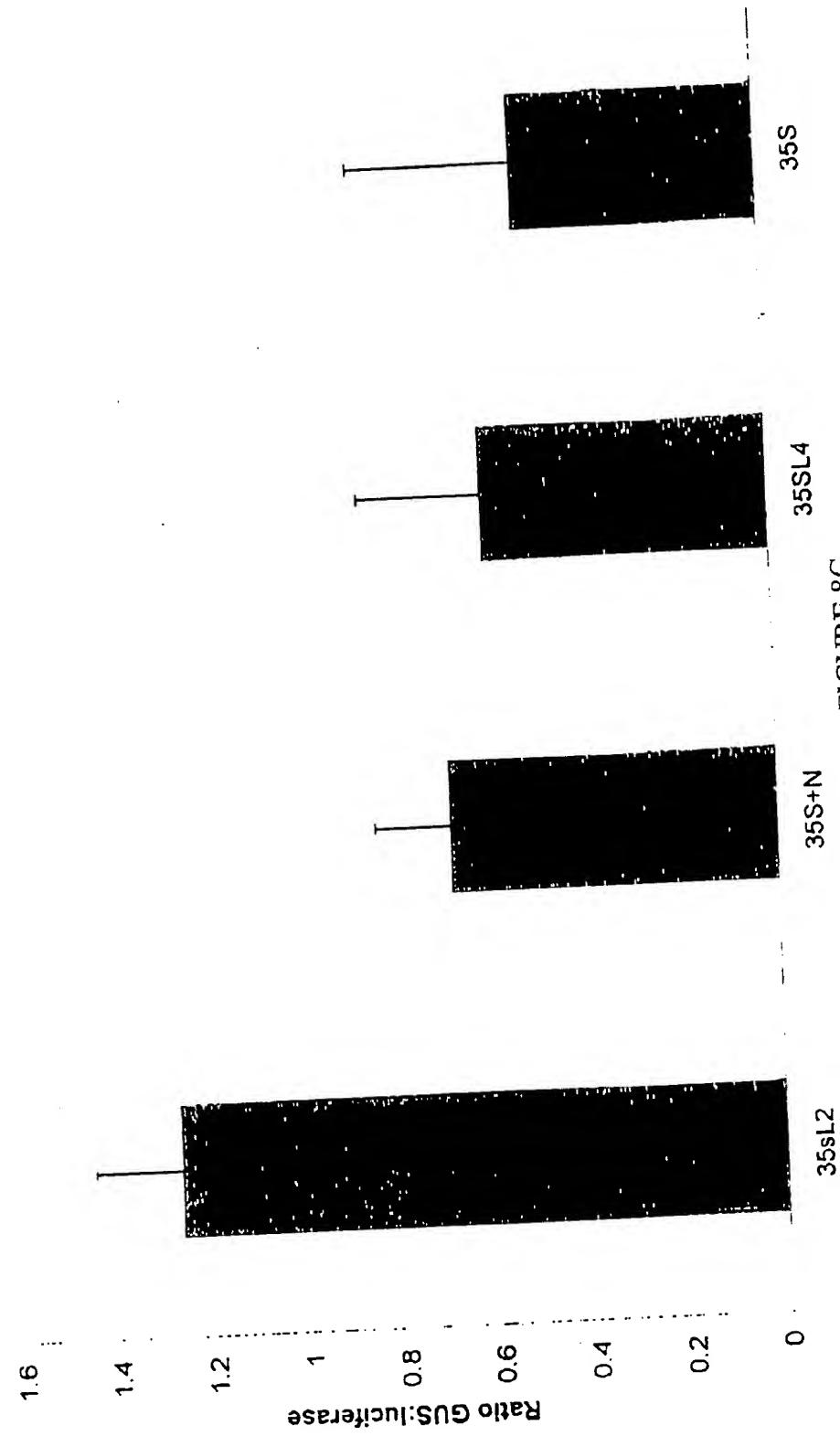


FIGURE 8C

Effects of L2 and L4 on 35S Tobacco Transient Assay

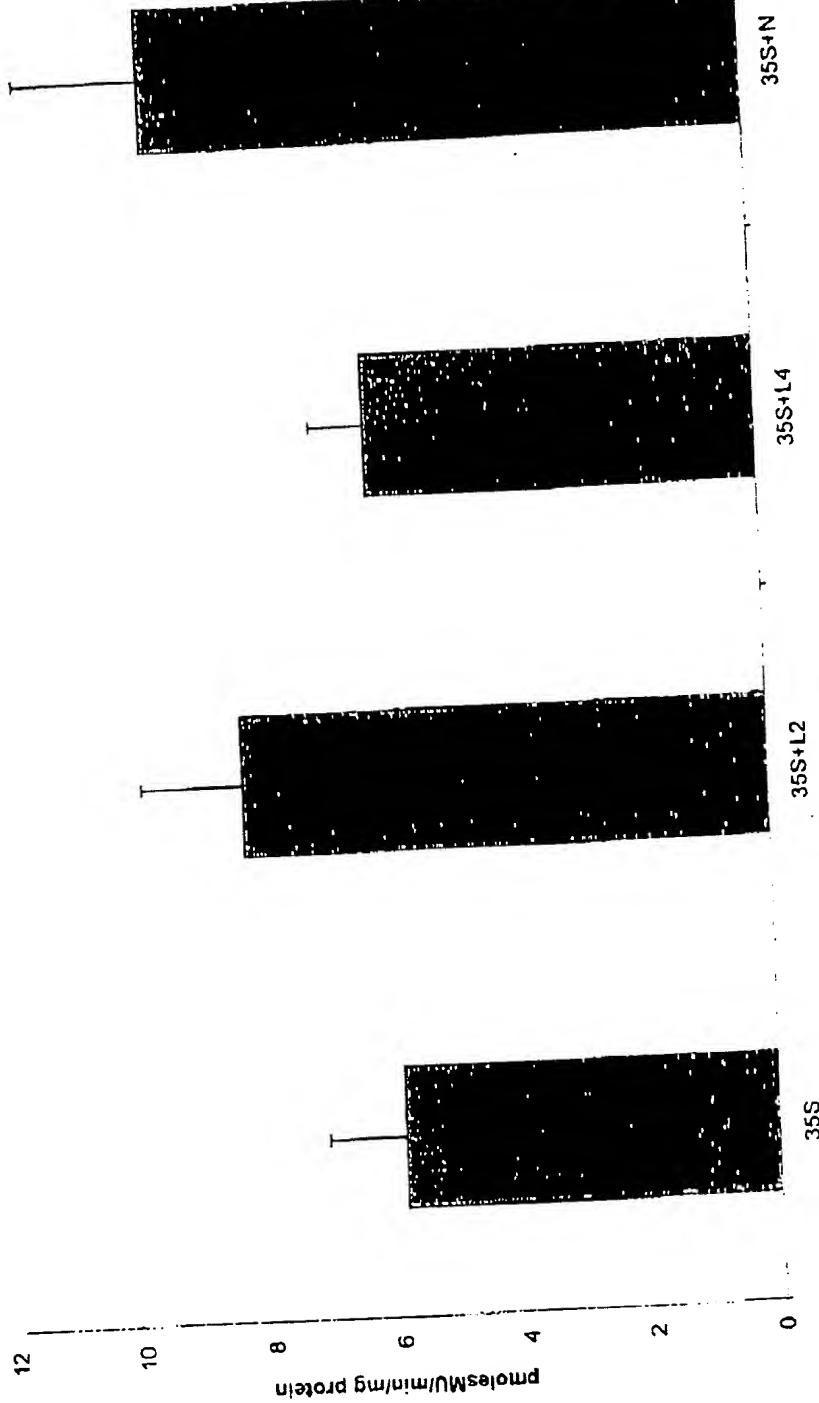
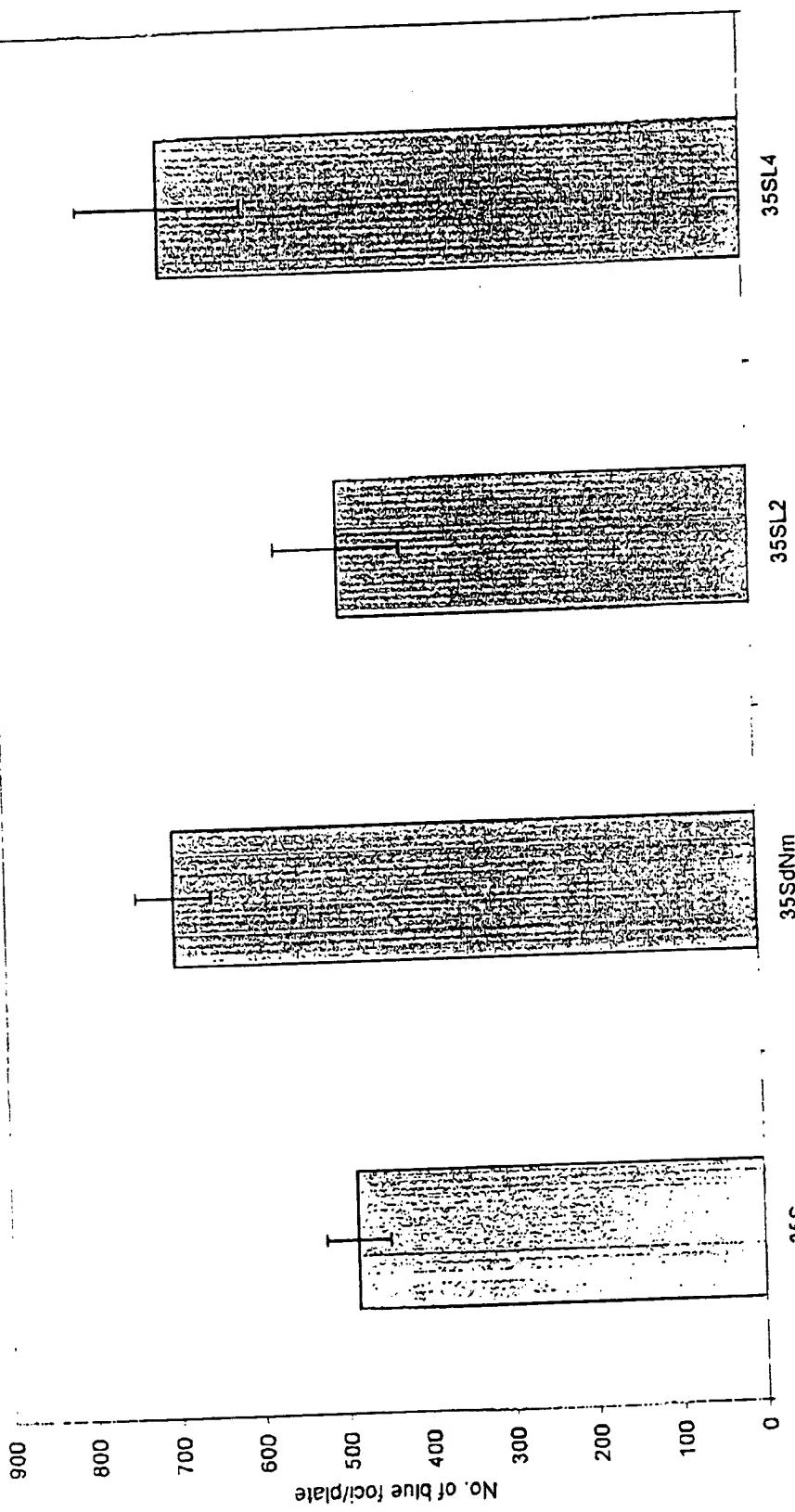


FIGURE 8D

Evaluation of the expression of tCUP leader and the elements, L2 and L4, with a heterogenous promoter (35S) in a transient GUS gene expression in alfalfa suspension culture



Construct
FIGURE 8E

0 50 100 150 200 250 300 350 400 450

500

400

350 300

250

200

150

100

50

0

Avg No. of Foci/plate

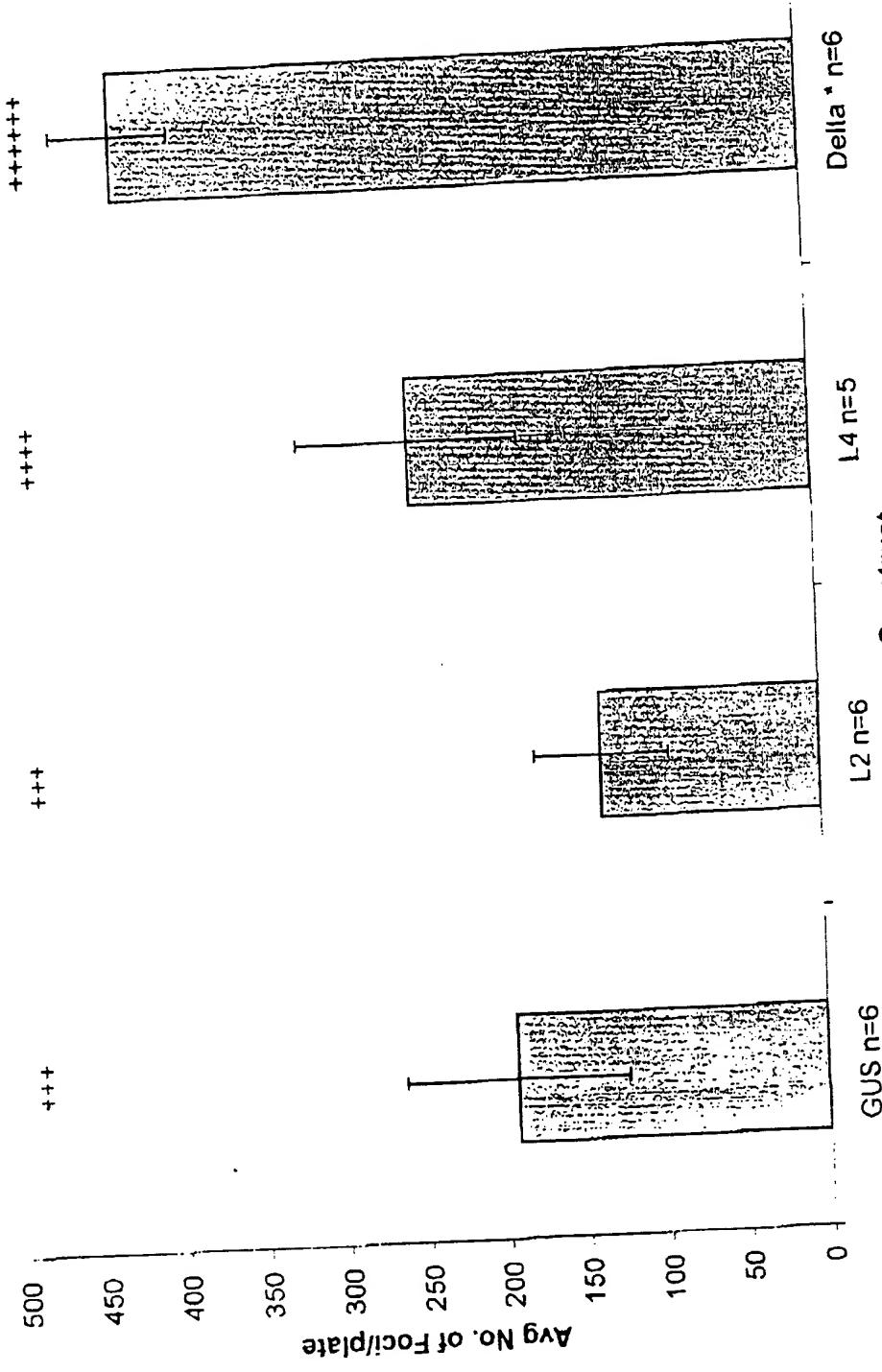
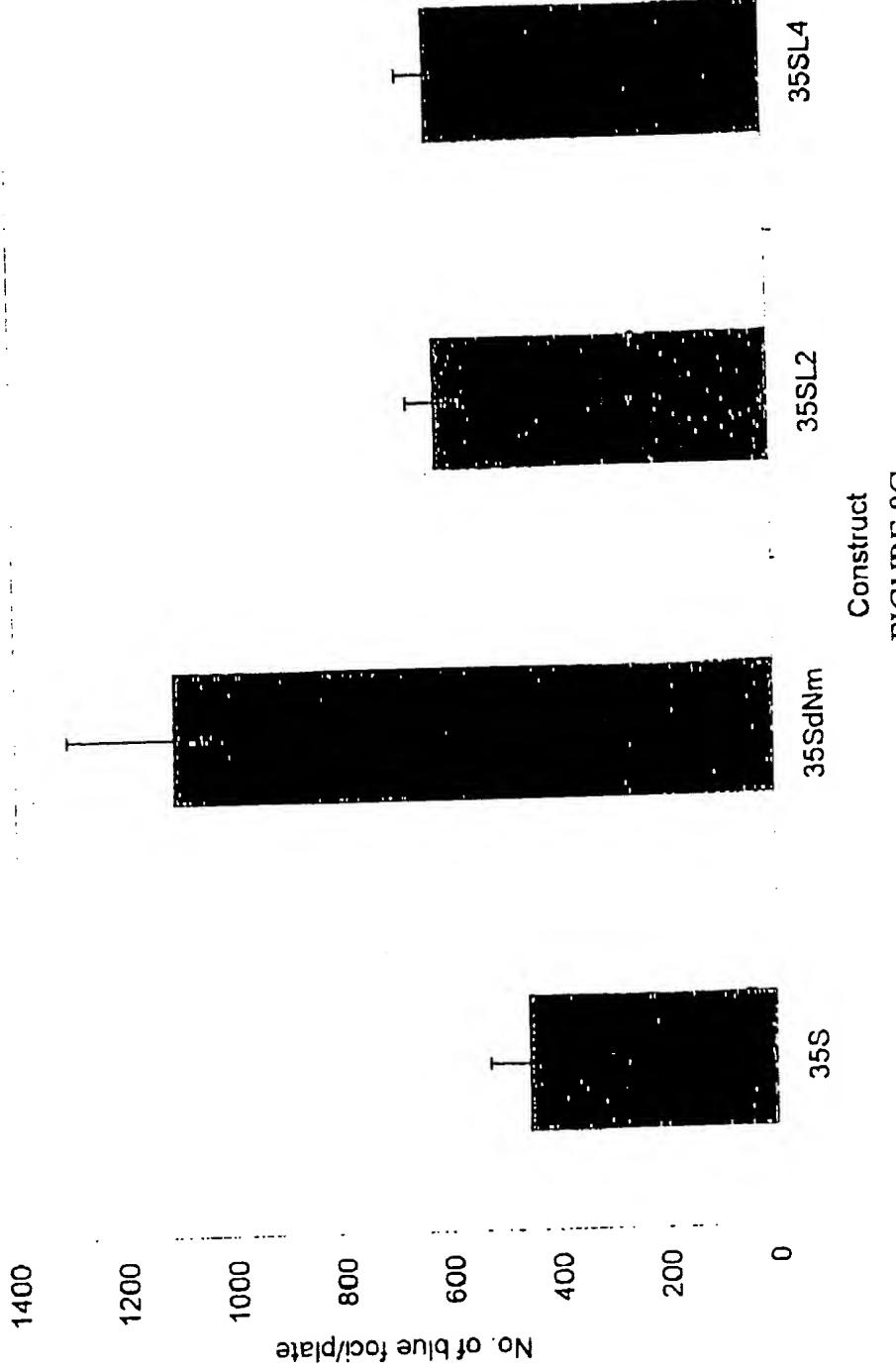


FIGURE 8F

Evaluation of the expression of tCUP leader and the elements, L2 and L4, with a heterologous promoter (35S) in a transient GUS gene expression in white spruce callus



Construct
FIGURE 8G

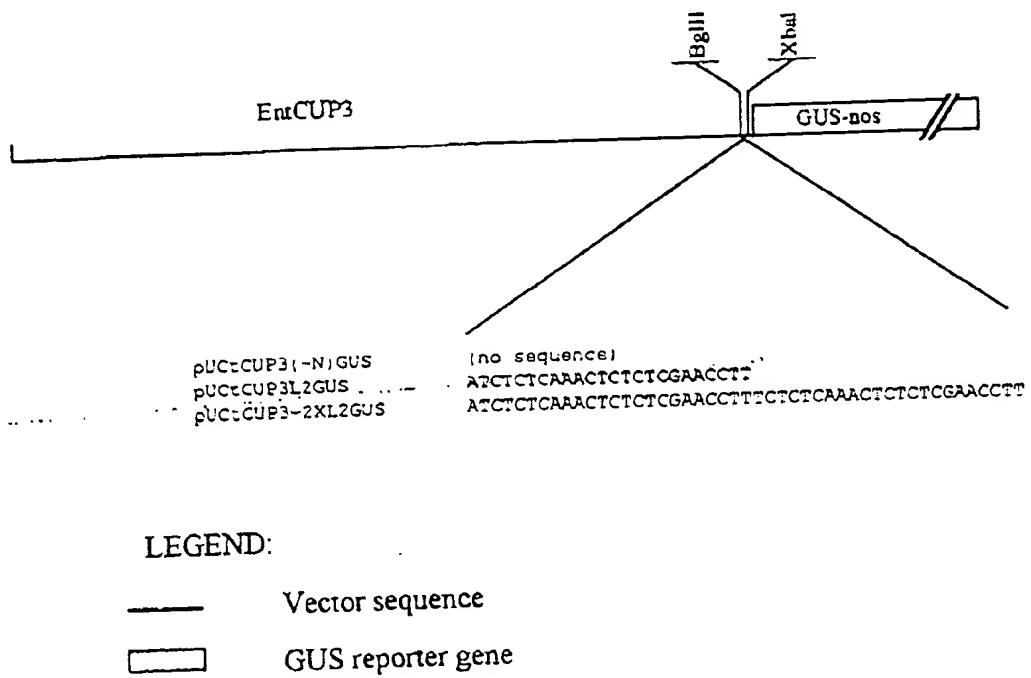


FIGURE 9A

GUS Expression of L2 Scan mutations and enh-tCUP2 in Tobacco
Transient Assay

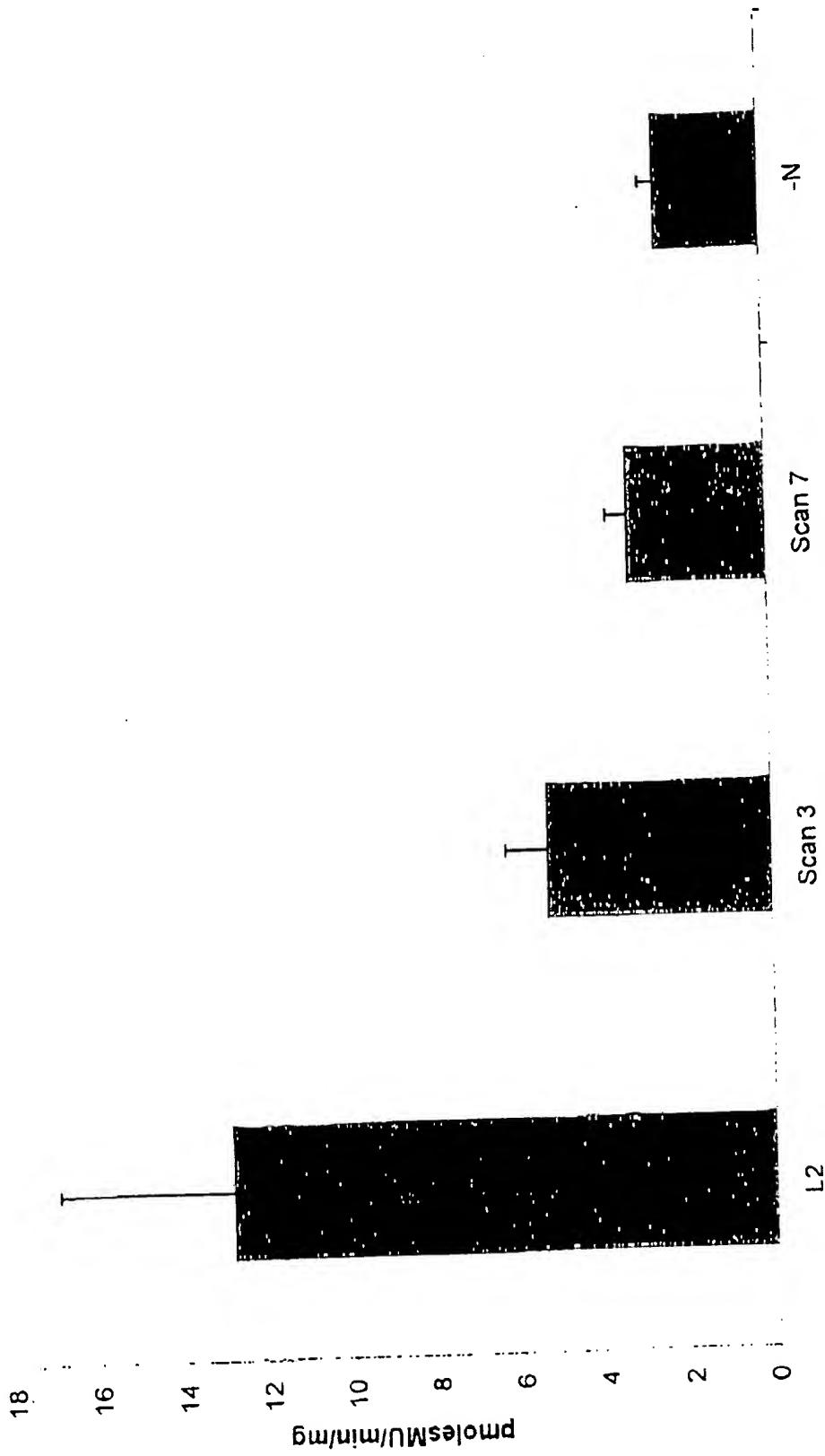


FIGURE 9B

Stable Transformation of *Arabidopsis* with GUS enhanced by L2 and 2XL2 constructs and the enh-tCUP2 and enh-tCUP3 promoter

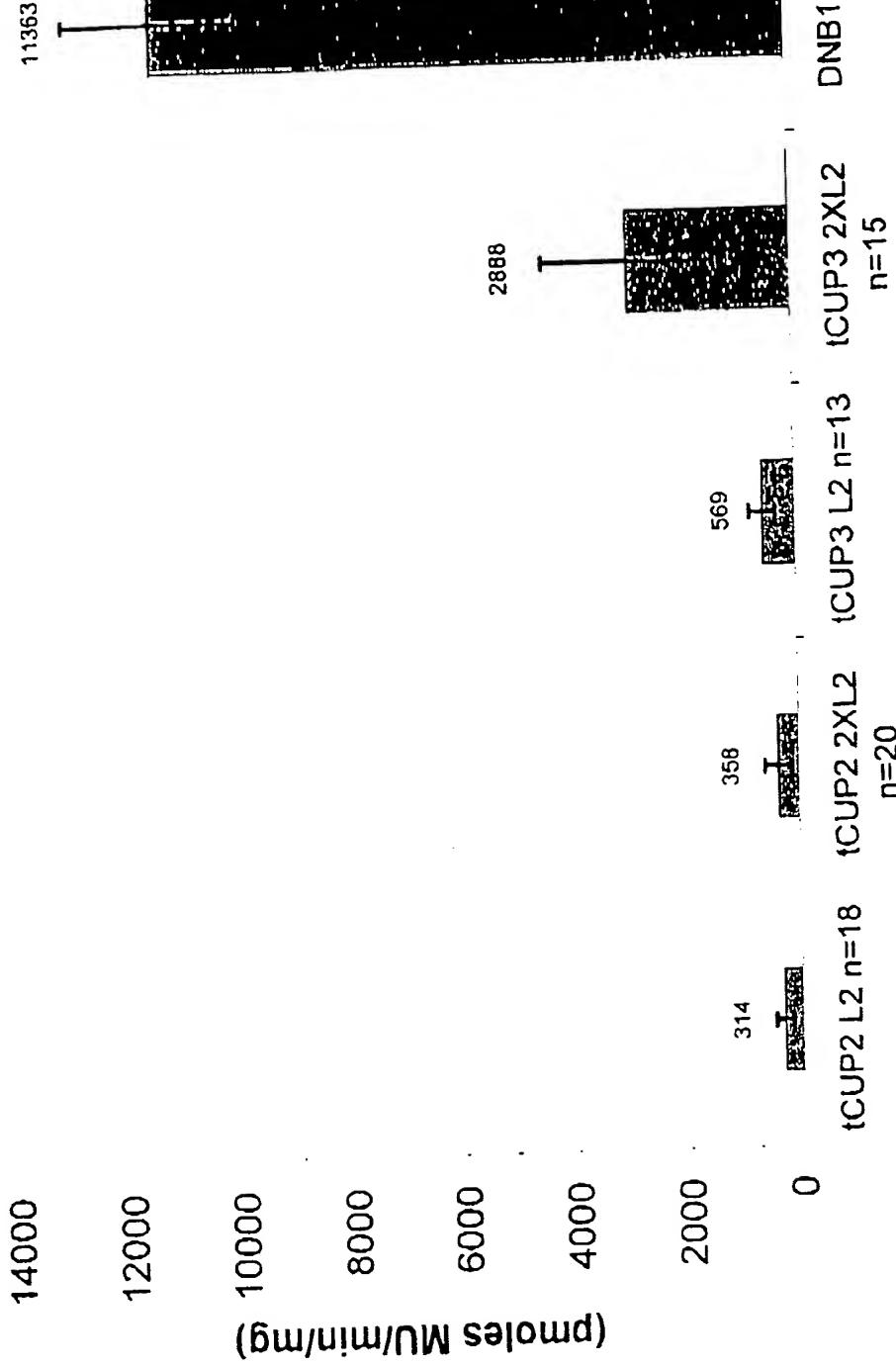


FIGURE 9C

Tobacco leaf bombardment of Enhanced tCUP vectors with L2

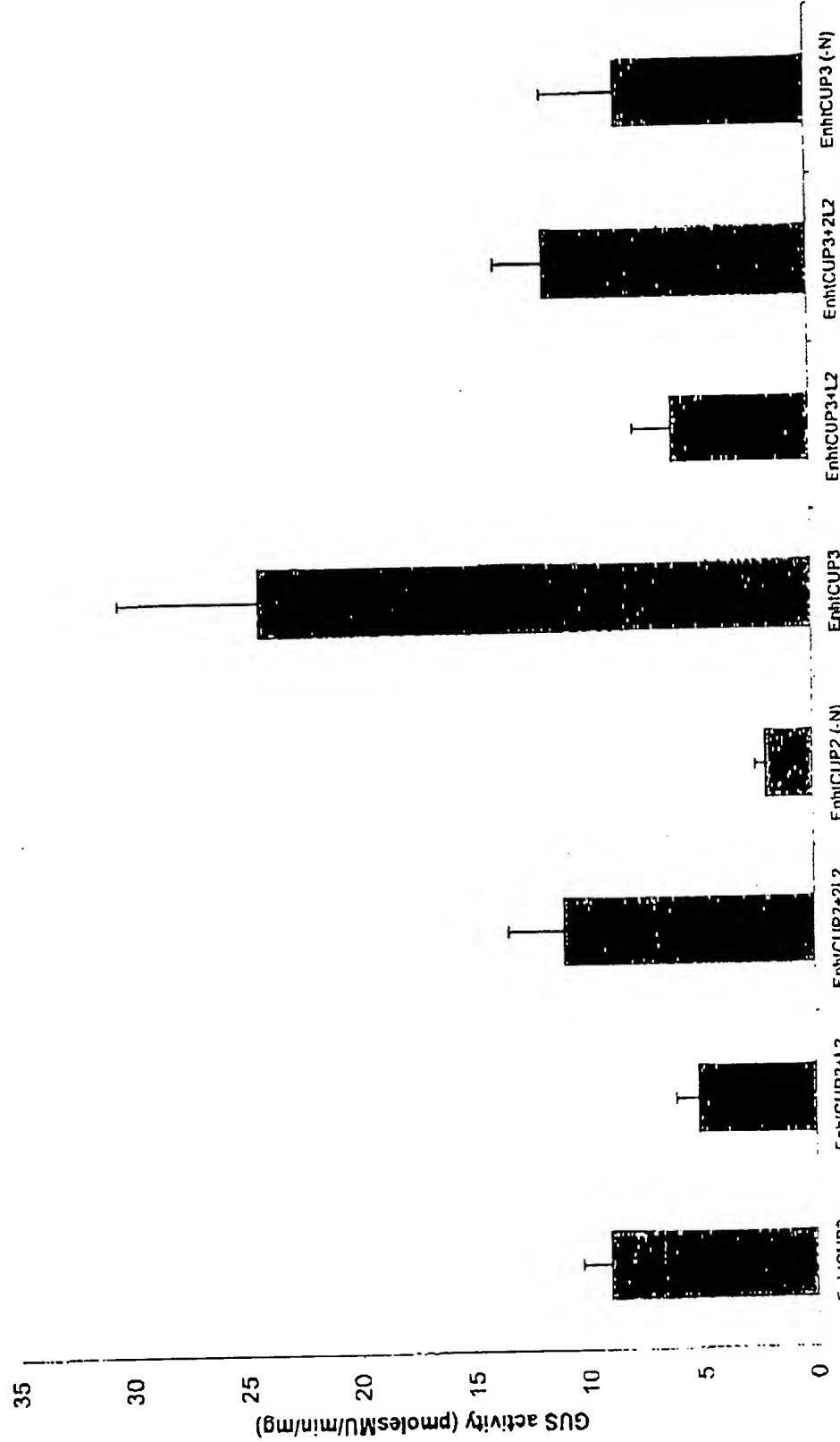


FIGURE 9D

Evaluation of tCUP leader element, L2, on transient GUS gene expression in *alfalfa* suspension culture

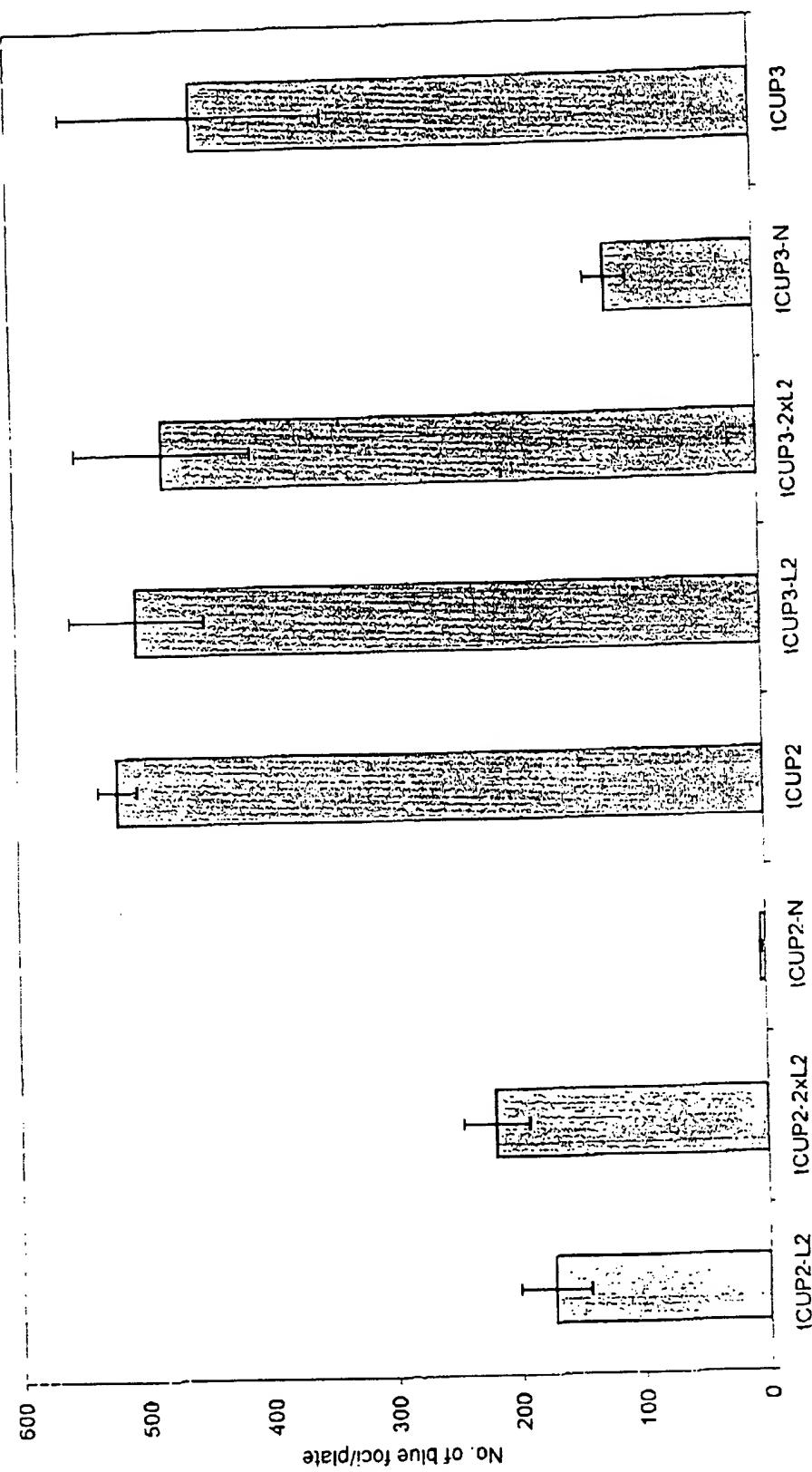


FIGURE 9E
Construct

Evaluation of tCUP leader element, L2, on transient GUS gene expression in
white spruce callus

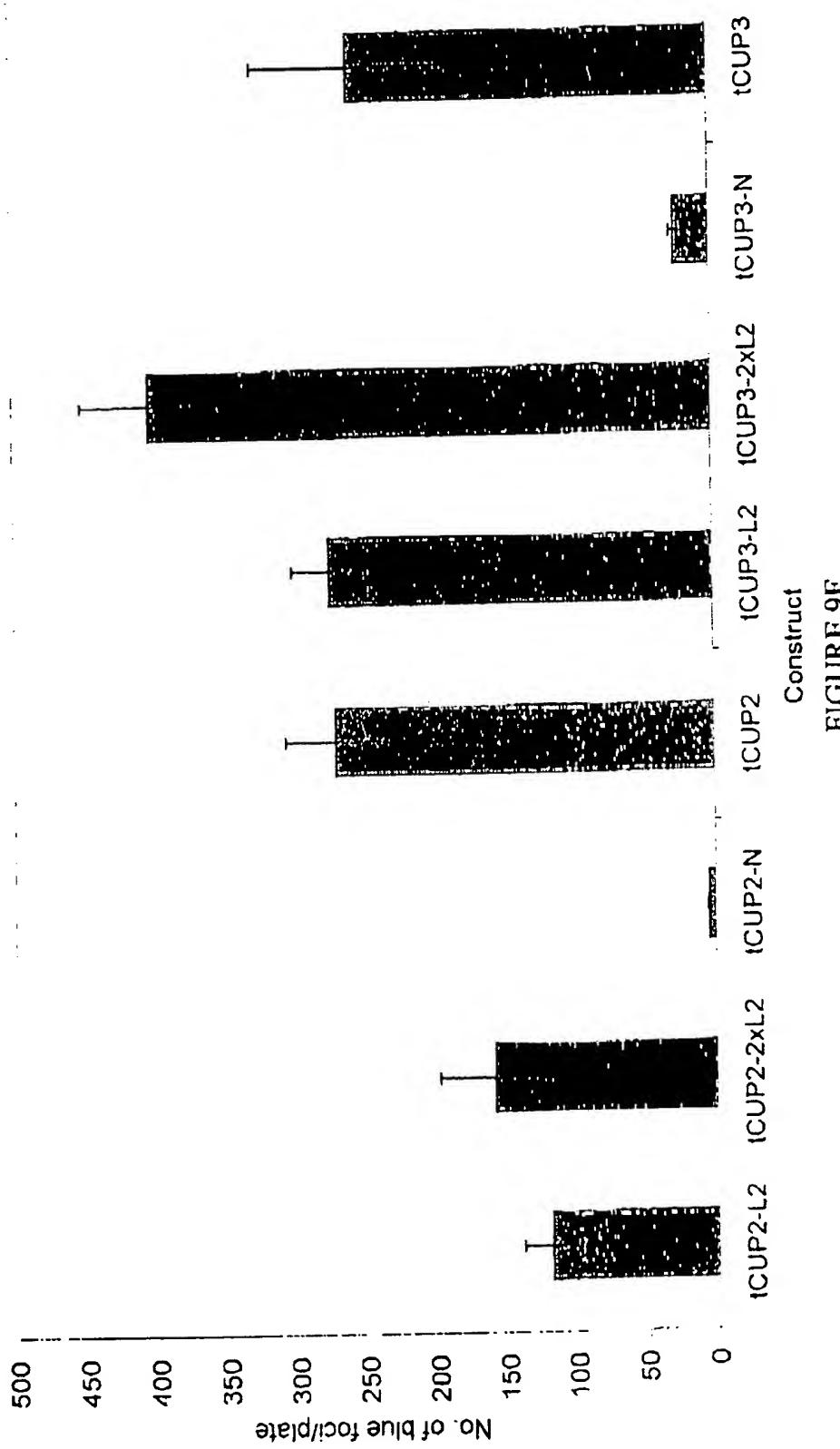
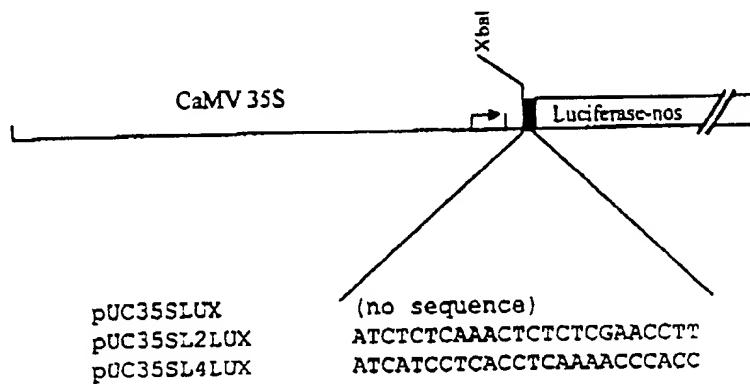


FIGURE 9F



LEGEND:

- Vector sequence
- Luciferase reporter gene
- L2 or L4
- Start of transcription

FIGURE 10A

Analysis of L2 and L4 in E. Coli using a luciferase reporter system

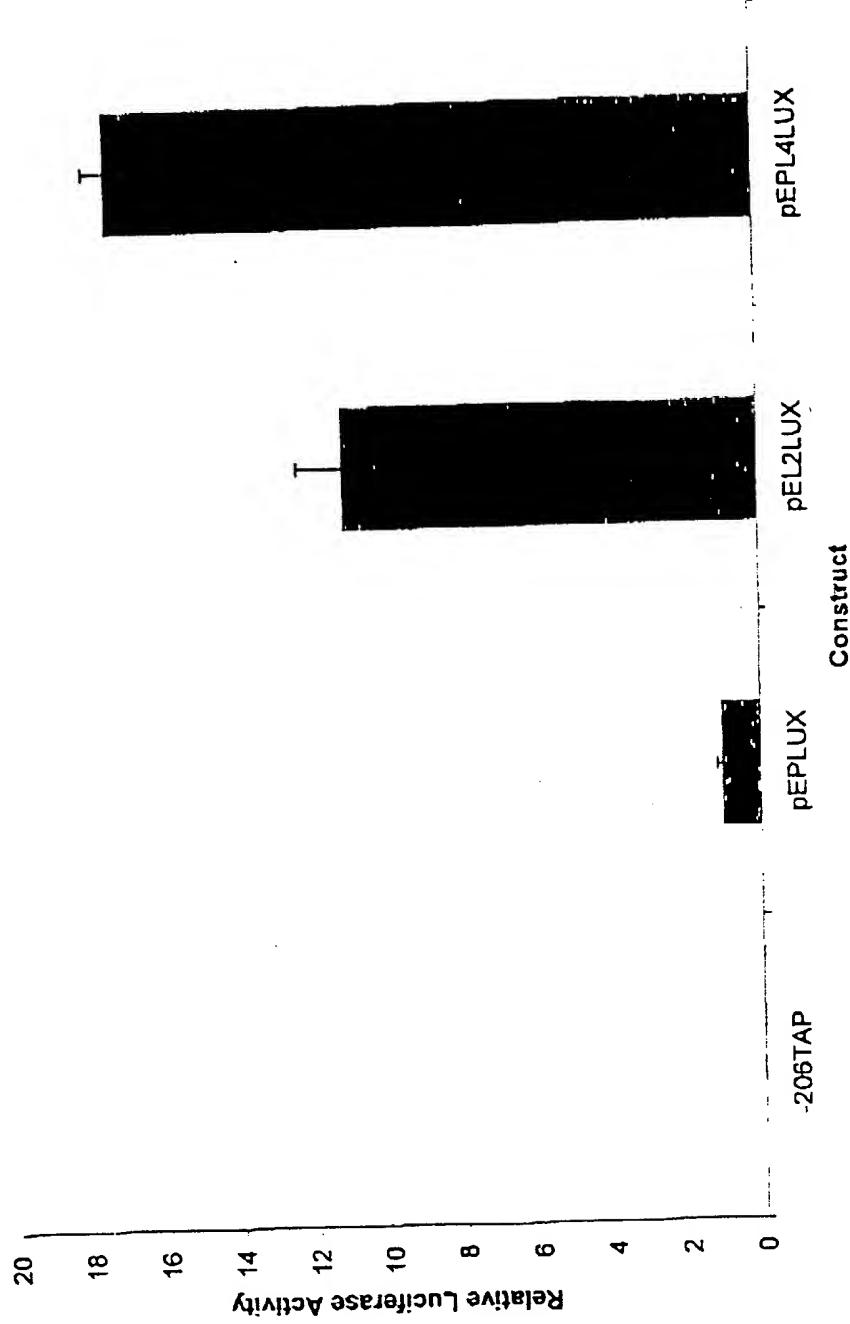


FIGURE 10B

© 1990 by the American Society for Cell Biology

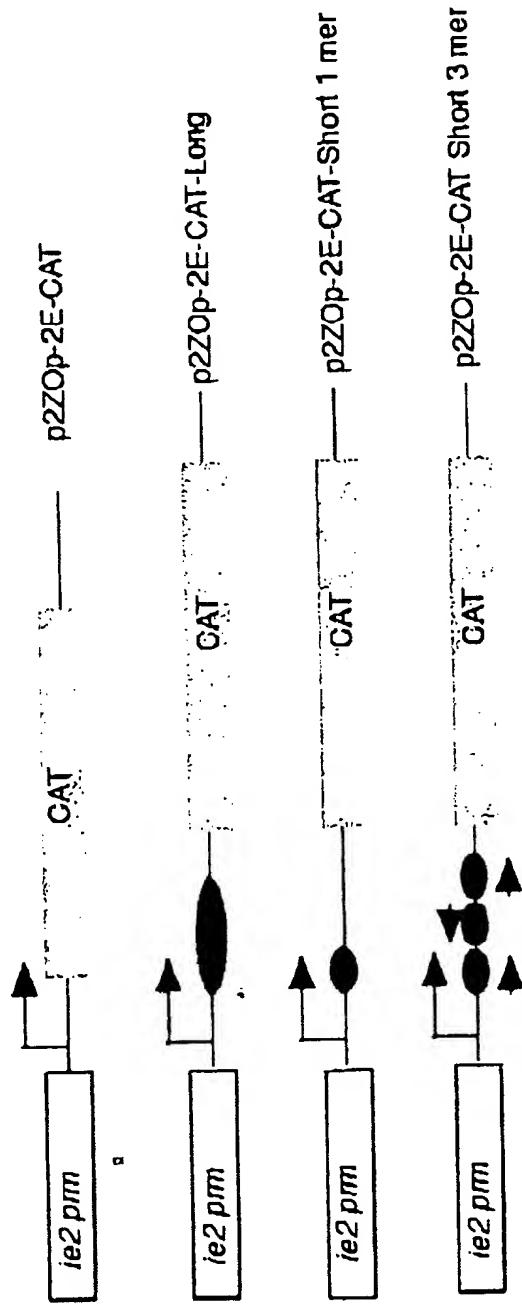


FIGURE 11A

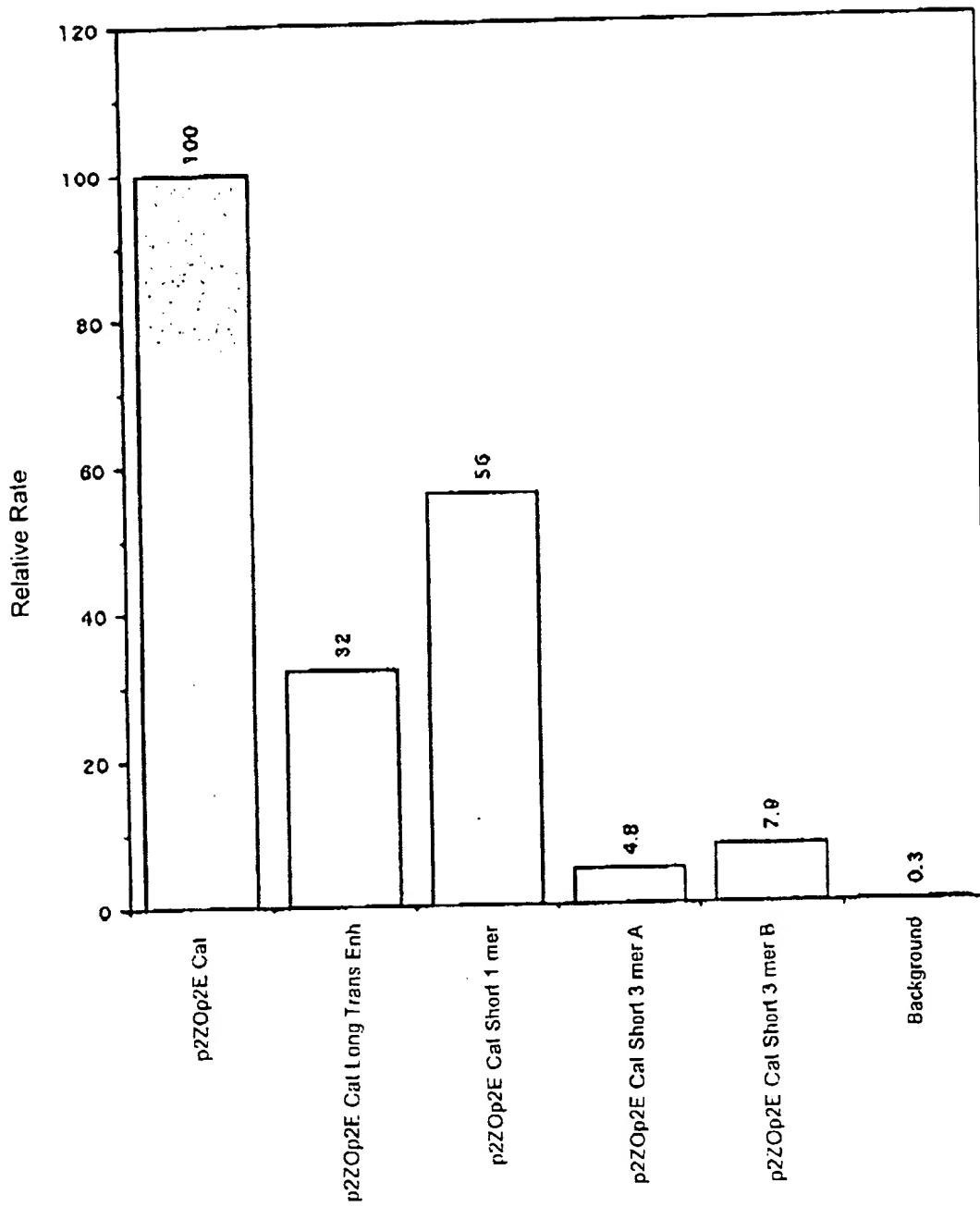


FIGURE 11B